PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

EDITED BY

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VOLUME 36, NOS. 1 & 2 SEPTEMBER 1944

RESPIRATORY SYSTEMS AND RESPIRATORY ADAPTATIONS IN LARVAE AND PUPAE OF DIPTERA

BY D. KEILIN, *Molteno Institute, University of Cambridge*

(With Plates I and II and 54 Figures in the Text)

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I. INTRODUCTION

Each year the study of Diptera reveals some new and often unexpected facts concerning the mode of life of their early stages. The rich entomological literature dealing with this subject clearly shows that the diversity of conditions under which the larvae of this order of insects live surpasses that of any other order of the Animal Kingdom.

There is hardly any type of medium capable of supporting life from which dipterous larvae have not been recorded. In fact, they occur in every kind of water media such as streams, rivers, waterfalls, ponds, lakes, brackish water, hot mineral springs and water reservoirs of terrestrial and epiphytic plants; they are found in a variety of dry and semi-fluid media such as sand, earth, mud, animal and vegetable substances in different degrees of decomposition, excrements of animals and infected wounds of animals and plants. They can be met with as scavengers in nests of mammals, birds, wasps, bees, ants and termites. As parasites they are found in different portions of plants, i.e. roots, stems, leaves and flowers, boring galleries or forming galls. They are known also to live as true parasites of a great variety of animals such as Oligochaetes, Molluscs, Crustacea, Arachnids, Myriapods, practically every large order of Insects, Amphibia, Reptiles, Birds and Mammals including Man. Finally, in a certain number of species the larvae grow to different stages of their development within the uterus of their mother, leaving it in some cases only just before pupation.

The great variety of conditions under which these larvae live, together with the frequent incompatibility of their nutritional and respiratory requirements has led to a very high degree of polymorphism, especially marked in the structure of their respiratory organs.

The problems which I propose to discuss in this paper can be grouped under two headings: (1) the study of the tracheo-spiracular system in dipterous larvae, and (2) the respiratory adaptations of these larvae to different media. The paper will be divided accordingly into two parts. The first part will comprise the study of the structure of the respiratory organs, and the structure of the perispiracular glands. In this part I propose also to deal with the distribution of different respiratory systems in different groups of larvae and the changes they undergo during the successive stages of their development. This will be followed by a study of the metamerism of the tracheo-spiracular system, the study of which will enable us to elucidate finally the question of the total absence of one pair of the thoracic spiracles in insects.

The second part of the paper will be devoted to the study of the respiratory adaptations of dipterous larvae and pupae to the great variety of external conditions which have been enumerated above. This study will reveal a series of interesting examples of convergency of certain structures in species phylogenetically distinct and divergency in forms nearly allied.

The paper will contain a brief discussion as to the possible lines of evolution of the respiratory system in the larvae of Diptera and will include a series of interesting examples illustrating the irreversibility of evolution.

I should like to add that this paper represents the results of work of more than 20 years and forms a part of a more ambitious plan of investigation covering both the morphological and physiological aspects of insect respiration. The biological and morphological study of the respiratory organs of larvae required, however, continual collection of fresh living material, long periods of observation, breeding of immature larvae, numerous dissections, reconstructions of complicated structure and numerous drawings. All this work absorbed much of my time and was continuous only during the first few years of this investigation. It began soon to suffer unavoidable interruptions increasing in frequency and duration, and had to be abandoned finally when certain purely biochemical aspects of insect respiration compelled me to enter a much wider, although intimately connected, field of research on intracellular respiration. The physiological and biochemical aspects of insect respiration have been, however, purposely excluded from the present study in the hope that they will be dealt with separately in papers published elsewhere.

Although the results of the work presented in this paper are far from being complete, yet I feel that even in their present form, they may be helpful to other workers in the study of the respiratory systems and adaptations in other orders of insects.

PART 1. RESPIRATORY SYSTEMS IN DIPTEROUS LARVAE

II. GENERAL CHARACTERS OF THE TRACHEAL SYSTEM IN DIPTEROUS LARVAE

The body of a dipterous larva is composed of a more or less free head, three thoracic and eight abdominal segments. Its tracheal system comprises the following parts (Figs. 1, 21 A and 22 G): (1) two main longitudinal latero-dorsal trunks (*Tr.*); (2) a pair of secondary latero-ventral longitudinal trunks (*t.l.*); (3) transverse branches (*b.t.*) which are given off laterally from the main tracheal trunks and connect it, in each segment, with the latero-ventral trunks; they continue as spiracular branches (*b.s.*) termi-

nating in more or less developed spiracles (*S.*); (4) transverse commissures (*cm.*) which connect, in each segment, the two main tracheal trunks; (5) tracheal branches or tufts, which arise principally

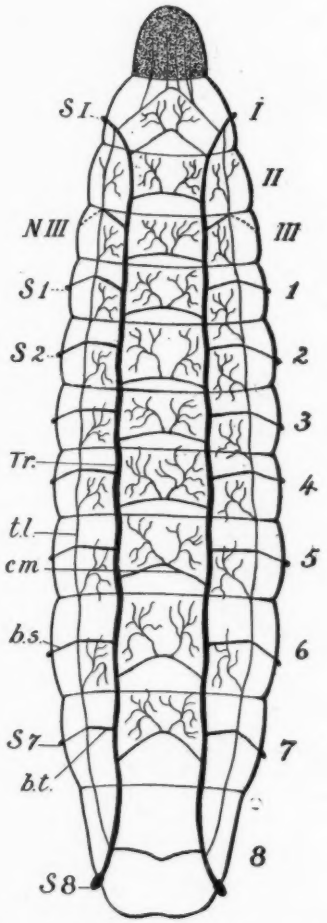


Fig. 1. Schematic figure representing the tracheo-spiracular system of a dipterous larva. *I, II* and *III*, the three thoracic segments; 1–8, eight abdominal segments; *S.* functional spiracles; *N.* non-functional spiracles; *b.t.* transverse branches; *b.s.* spiracular branches; *cm.* transverse commissures; *t.l.* latero-ventral longitudinal trunks; *Tr.* main tracheal trunks.

from the secondary latero-ventral trunks, the ramifications of which extend to various organs; (6) branches which arise from transverse commissures; (7) spiracles, functional or non-functional, and perispiracular glands.

The numerous variations of the respiratory system in dipterous larvae and their more detailed structure will be dealt with in the sections which follow. It can be mentioned here that with a few exceptions the structure of the tracheae in dipterous larvae does not differ from that of other insects. The epithelial layer or ectotrachea is the continuation of the hypodermal layer of the body wall, just as the chitinous intima or endotrachea is continuous with the cuticle of the body. The tracheal intima shows a characteristic ridge-like spiral thickening projecting into the lumen of the tracheae and known as the taenidia. In a few cases only the usual spiral structure is replaced either by a wide spiral band giving to the trachea a peculiar concertina-like appearance as in the case of *Phaonia mirabilis* (Fig. 2 A) or by a bead-like structure found in some tracheae in the same species (Fig. 2 B, C) and in the larva of *Hypoderma lineata* (Fig. 2 D, E). In the larvae of *Gastrophilus* one of the trachea suddenly widens into a small tracheal body (Fig. 2 F, G, *T.b.*) in which the spiral taenidia are replaced by chitinous reinforcements distributed in a characteristic pattern.

That the taenidia are produced by the protoplasmic activity of endothelial cells of tracheae in the same way as the cuticular ornamentations of the body are produced by the hypodermic cells can be clearly seen in the tracheae during the process of moulting (Fig. 2 H, I).*

III. STRUCTURE OF SPIRACLES AND THE PROCESS OF THEIR MOULTING

(a) *Three types of spiracles*

Practically all the information which we possess concerning the structure of spiracles in dipterous larvae is scattered in numerous papers dealing with different aspects of larval morphology. The account and figures given in the present paper are based, however, mainly on personal observations completed by only such information from the literature which clearly illustrates the structural variation of spiracles in this order of insects.

The structure of spiracles is much more easily understood when they are studied during the process of moulting, which separates two successive larval stages. Such a study can best be carried out on comparatively transparent living larvae compressed between a slide and cover-slip. We owe to de Meijere

* This observation disproves the theory of the origin of the spiral threads proposed by Packard (1886 and 1909, see p. 449 and Figs. 412, 413) who believed that 'the so-called "spiral thread" is formed by thickenings of the nuclei composing the secondary layer of nuclei, and which become filled with the chitin secreted by these elongated nuclei'.

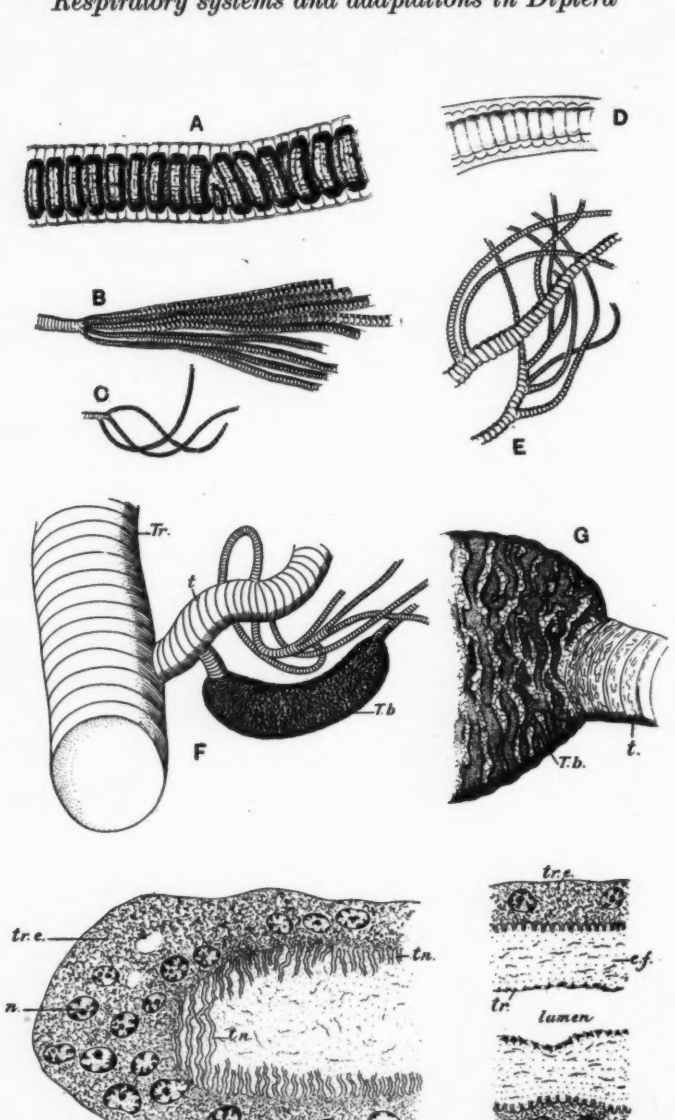


Fig. 2. A, tracheae with wide spiral band in the larva of *Phaonia mirabilis* Ringdahl; B, C, bead-like tracheae in the same larva; D, E, bead-like tracheae in larvae of *Hypoderma lineata*; F, G, tracheal body (*T.b.*) larva of *Gastrophilus intestinalis* arising from a tracheal branch (*t.*) connected with tracheal trunks (*Tr.*); H, I, longitudinal sections of tracheae in *Oestrus ovis* during the moult separating the second and the third larval stages. *e.f.* ecdysial fluid; *n.* nuclei of the tracheal epithelium; *tn.* taenidia in formation; *tr.* tracheal intima of the second stage; *tr.e.* tracheal epithelium secreting the third stage tracheal intima. A and F × 65, B–E and G–I × 300.

(1894) the first correct description and interpretation of the process of moulting in different types of spiracles. In the course of the present study the main results obtained by de Meijere were confirmed, and the terminology introduced by him for different structures was adopted.

The spiracles in dipterous larvae, in spite of their great structural variations, can be separated into three main types. These types are not sharply defined as there are numerous intermediate forms which make one type merge gradually into the other. The main characters of these types can be defined as follows:

Type I. Spiracles in which the ecdysial opening becomes the spiracular opening of successive stages.

Type II. Spiracles in which the ecdysial opening is obliterated and a spiracular plate with fine radial clefts or papillae with clefts are formed around the ecdysial scar which is more or less central.

Type III is similar to type II except that the spiracular plate lies outside the ecdysial scar and does not surround it.

In all three types the tracheal trunks or spiracular branches are connected with the spiracles by means of a short intermediate portion devoid of spiral rings. This portion forms a spiracular chamber, the lumen of which is either free or is filled with a more or less dense chitinous felt forming a very efficient filter. This intermediate portion, even when its lumen is comparatively free of felt, will be referred to as the felt chamber of the spiracle.

(*b*) *Moulting process of spiracles*

This process will be examined separately for each of the three spiracular types.

Type I. To this type belong the postabdominal spiracles of mosquito larvae. Each spiracle is represented by an opening on the 8th abdominal segment extending in some cases in the form of a more or less developed siphon. The spiracular opening is connected with the corresponding tracheal trunk by means of an intermediate piece almost free of felt.

The moulting of such a tracheo-spiracular organ is very simple. The hypodermal layers of cells proliferate and secrete the new tracheo-spiracular system which is in continuation with the new cuticle of the body and which surrounds the old structures, being separated from them by a wide space filled with ecdysial fluid. When the moult is completed the old cuticle is shed and the old spiracles attached to it, together with its felt chamber and tracheal trunks, are expelled through the new spiracular aperture (Fig. 3 A, B).

Type II. The spiracle of this type, schematically represented in Fig. 4 A–D, consists of a spiracular plate, the central portion of which is strongly sclerotized and often protrudes into the felt chamber. The peripheral portion of the plate is covered by a thin membrane reinforced by chitinous rods and showing fine radiating spiracular clefts which represent the spiracular openings. The central sclerotized area as shown in Fig. 4 A–D represents only a scar plug (*Sc.p*) which is formed by the contraction and hardening of the chitin surrounding the ecdysial opening through which is expelled the tracheo-spiracular system of the previous larval stage. The opening thus becomes obliterated. This type of spiracle occurs in the larvae of Tipulidae, Trichoceridae and other families of Diptera.

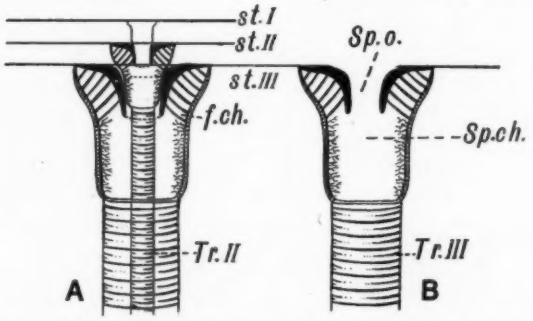


Fig. 3. Schematic figure of the moulting process of an open spiracle of type I; A, during, and B, after, the moult. *Sp.o.* spiracular opening. For explanation of other letters see Fig. 4.

Type III. The simplest examples of this type are shown by the postabdominal spiracles of larvae of cyclorrhaphous Diptera (Fig. 4 E–H). Such a spiracle consists generally of a sclerotized chitinous plate with three elongated depressions (*Sl.*) covered by a membrane bearing longitudinal slits and supported by transverse chitinous rods. These three depressions, described usually as spiracular openings, converge towards a circular spot (*e.sc.*) lying near the internal border of the spiracular plate (Fig. 4 H).

Examined from the side (F), the spiracle shows that the tracheal trunk communicates with the spiracular plate by means of a well-developed felt chamber (*f.ch.*). The latter is wide at its basal portion and becomes narrower as it approaches the spiracle, dividing into three flattened branches which terminate in the three elongated depressions. Moreover, the circular spot (*e.sc.*) of the spiracular plate is connected with the basal part of the felt chamber by a colourless strand (*Sc.f.*) or cord of chitin which is inserted into a circular pit (*i.sc.*) in the wall of the chamber. The significance of certain structures in this type of spiracle is more easily understood when the spiracle is examined during the process of moulting which separates the second and third larval stages (Fig. 4 E, G). It can then be seen that the felt chamber of the second stage larva with its spiracular plate (*S.st.II*) emerges from the spiracular plate of the third stage (*S.st.III*), through a large external ecdysial opening (*e.e.o.*). The proximal part of the tracheo-spiracular system of the second stage is still seen to be contained within the body of the larva. There it is seen to be surrounded by a delicate chitinous ecdysial tube (*ec.t.*) which extends from the external ecdysial opening (*e.e.o.*) to the internal ecdysial opening (*i.e.o.*) lying on the dilated portion of the felt chamber. It should be noted that the ecdysial tube separates the ecdysial fluid, filling the space between the chitinous structures of the two stages, from the perivisceral fluid of the larva. This tube thus effects the continuity of the ecdysial fluid which fills the tracheal system of the third stage larva, with that which is present in the space separating the cuticles of the second and third stages of the larva.

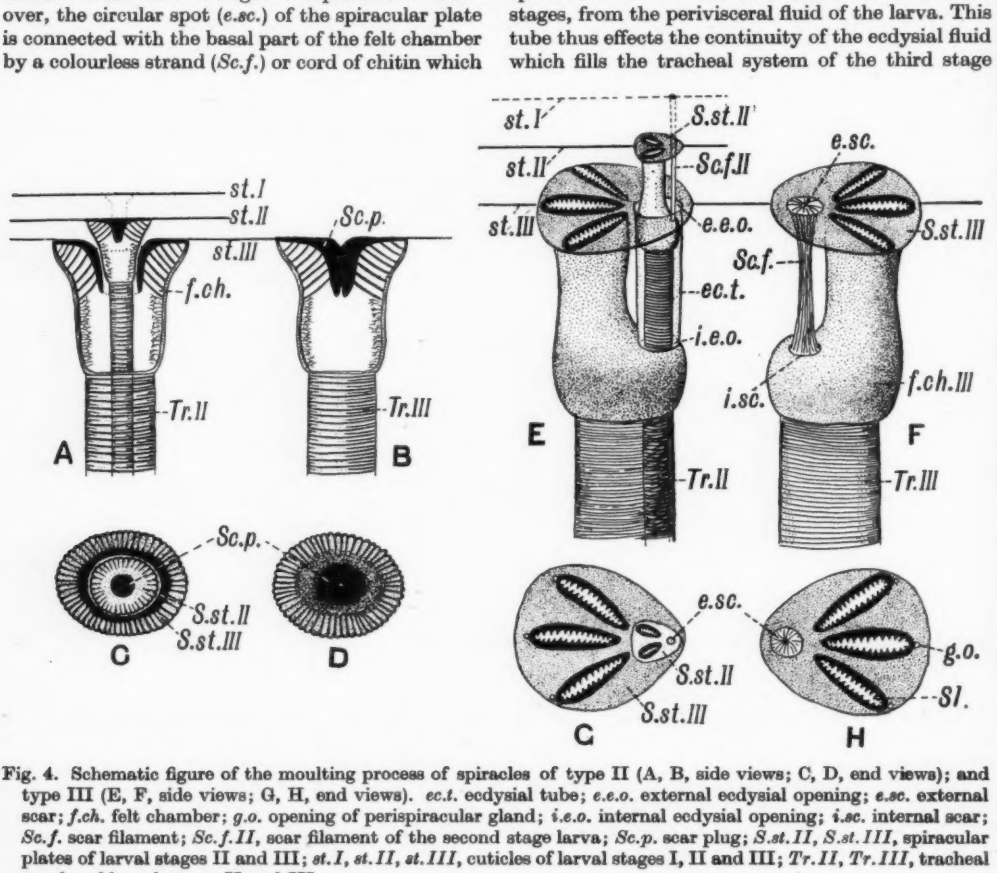


Fig. 4. Schematic figure of the moulting process of spiracles of type II (A, B, side views; C, D, end views); and type III (E, F, side views; G, H, end views). *ec.t.* ecdysial tube; *e.e.o.* external ecdysial opening; *e.sc.* external scar; *f.ch.* felt chamber; *g.o.* opening of perispiracular gland; *i.e.o.* internal ecdysial opening; *i.sc.* internal scar; *Sc.f.* scar filament; *Sc.f.II*, scar filament of the second stage larva; *Sc.p.* scar plug; *S.st.II*, *S.st.III*, spiracular plates of larval stages II and III; *st.I*, *st.II*, *st.III*, cuticles of larval stages I, II and III; *Tr.II*, *Tr.III*, tracheal trunks of larval stages II and III.

With the completion of the moult (Fig. 4 F, H) the second stage tracheal system is completely removed from the third stage trachea. The ecdysial tube now collapses and is transformed into the scar filament (*Sc.f.*), while the two ecdysial openings close and leave the corresponding external (*e.sc.*) and internal (*i.sc.*) scars. The moult which separates the first and the second larval stages follows a precisely similar course.

(c) *Variations in structure of spiracles*

The structure of spiracles in dipterous larvae varies not only with the species of the larva but often with their position on the body. Thus the same larva may show three different kinds of spiracles, one kind on the prothoracic segment, the other on the last abdominal segment and the third on other segments of the body. It is usually the prothoracic and the postabdominal spiracles that show a marked difference in their structure. Such polymorphism of spiracles is often intimately connected with the respiratory adaptation of the larva to surrounding conditions and will therefore be dealt with in a chapter specially devoted to this subject.

We shall now examine briefly a few examples of spiracles which will illustrate the main variations in their structure.

(1) *Ctenophora ornata* Mg.

This larva, like those of other true tipulides, is metapneustic, i.e. it has only one pair of spiracles which belong to the last abdominal segment. The spiracles of these larvae have already been described and figured by Brown (1910) in the larva of *Tipula maxima*. Although his description is the best available for this group of larvae, it can be completed by certain important characters observed in the larvae of *Ctenophora*.

The spiracular plate of *Ctenophora* (Fig. 5 A–D) belongs to the second type of spiracles described above. It is a circular plate which is divided into two distinct regions (C): a peripheral region marked by numerous closely set sclerotized radiating ribs which give to this region the appearance of being striated, and a central region formed of undifferentiated chitinous structure highly sclerotized near its middle. The central region represents the scar plug (*Sc.p.*) which can also be seen on the section passing across the plate (A, *Sc.p.*).

The structure of the peripheral portion can best be seen on microscopic examination of sections which are slightly oblique to the surface of the plate. Such sections show (B, D) that the surface of this portion is covered by a thin chitinous membrane perforated by long longitudinal slits (*sl.*) converging toward the centre of the spiracular plate. This membrane is reinforced by a series of chitinous radiating rods (*ch.r.*) giving off laterally fifteen pairs of short chitinous projections in the form of teeth. These structures are connected in deeper layers with numerous vertical rods (*v.r.*) and other chitinous strands and filaments forming a real felt (*f.*). The space under the spiracular plate forms a large spiracular chamber (*Sp.ch.*) with a felt of chitinous filaments arising from the internal wall of the chamber but not reaching its centre which is free from the felt. The air reaches this chamber by passing through the longitudinal radiating clefts of the spiracular plate and the felt lying just beneath the plate (A, *f.*). A very similar spiracular structure is found in larvae of most of the other tipulides.

(2) *Trichocera hiemalis* De Geer

The structure of the postabdominal spiracle in this larva resembles that of *Ctenophora*. The main difference consists in a much larger and thicker scar plug projecting into the lumen of the felt chamber (Fig. 6 A, B). The peripheral region of the spiracular plate does not show the radial chitinous rods, and the numerous slits in the chitinous membrane can be seen only with strong magnification. A section across the spiracle shows often the presence of a distinct slit in the scar plug which seems to connect the spiracular chamber with the external air. This slit is an artefact produced during the sectioning and represents probably the remains of an obliterated ecdysial tube filled with less hardened chitin which ruptures during the process of sectioning.

(3) *Laphria* and *Thereva*

The prothoracic spiracle of *Laphria* (Fig. 7 A, B) is in the form of a semicircular plate showing about eighteen short sclerotized rods supporting the anterior border of the plate; the posterior border shows a depression corresponding to an external scar connected with a scar plug which, as a dark chitinous mass, can be seen by transparency lying within the felt chamber (*f.ch.*). The spiracular plate is covered by a thin chitinous membrane (*mb.*) showing about eighteen small peripheral slits (*sl.*) lying above and between the peripheral rods. The felt chamber appears to be filled with numerous chitinous projections originating from its walls and directed towards the scar plug. The spiracles of *Thereva* show a somewhat similar structure. The prothoracic spiracle has only two slits (C, *sl.*) and the postabdominal eight slits (D, *sl.*) in the thin membrane (*mb.*) covering the plate. The slits in both cases lie between strong peripheral chitinous projections directed towards the external scar and the scar rod (*Sc.r.*).

(4) *Certain cyclorrhaphous Diptera*

The third type of spiracle which we have previously examined is very common among the larvae of cyclorrhaphous Diptera such as anthomyids, calliphorines, sarcophagines, tachinids and many families of acalypterates. In certain forms the peritremes bordering the spiracular clefts are bent or even sinuous, as is the case of *Musca domestica* (Fig. 8 A, B). In other larvae, as was shown by Nielsen (1909, 1916), the peritremes break up into smaller portions of unequal size (Fig. 8 C, D) or into numerous papillae (E, F).

(5) *Oedomagena tarandi* L.

The postabdominal spiracles of a full-grown larva of this oestrid are oblong (Fig. 9 A) and show numerous papillae (*p.*) distributed in more or less regular groups spreading fan-like from the external scar (*e.sc.*) towards the periphery. The scar is almost completely enclosed within the spiracular plate which is bent around it, leaving only a small region of undifferentiated chitin near the middle of its internal border. A series of sections parallel to the surface of the plate (Fig. 9 B–D) shows that the papillae originate from folded and ramified branches of the felt chamber (*f.*) which opens internally into a spiracular chamber almost completely free of felt. These sections show also that a certain portion of the scar cord (*Sc.cd.*) just beneath the surface of the spiracular plate lies free within the bend of the kidney-shaped section of the felt chamber (B, C).

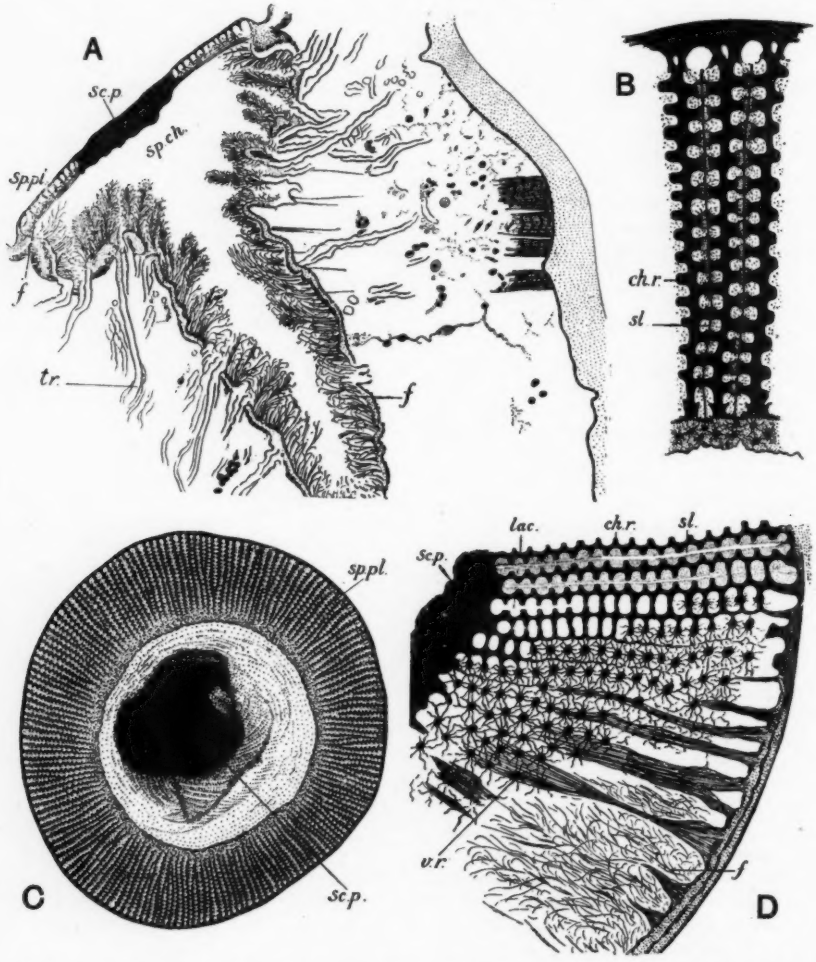


Fig. 5. Postabdominal spiracle of a full-grown larva of *Ctenophora ornata* (Tipulidae). A, longitudinal section across spiracular plate and tracheal trunk; B, surface section of the spiracular plate; C, surface view of spiracular plate; D, slightly oblique section of spiracular plate showing chitinous structure at different levels. *ch.r.* chitinous rods; *f.* felt chamber; *lac.* lacunae; *sl.* slit; *Sc.p.* scar plug; *Sp.ch.* spiracular chamber; *sp.pl.* spiracular plate; *tr.* tracheoles; *v.r.* vertical rods. A and C × 90, B × 600, D × 350.

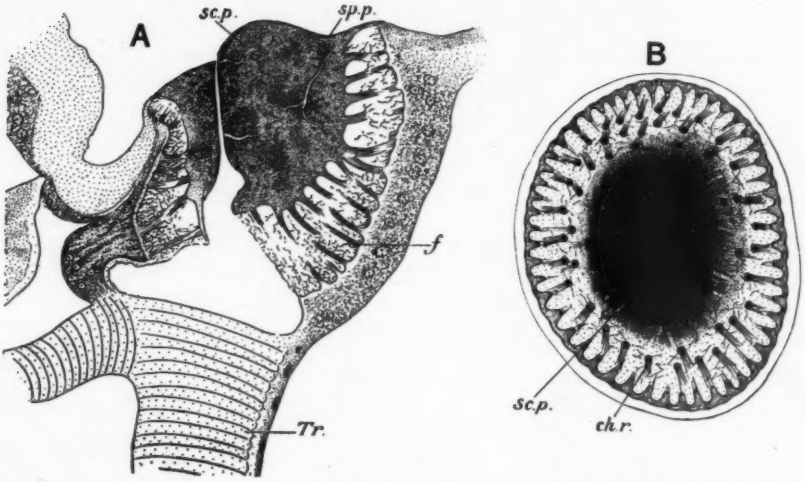


Fig. 6. Postabdominal spiracles of the larva of *Trichocera hiemalis*. A, longitudinal section; B, end view of spiracular plate. *Tr.* tracheal trunks. Other letters as in Fig. 5. A and B × 600.

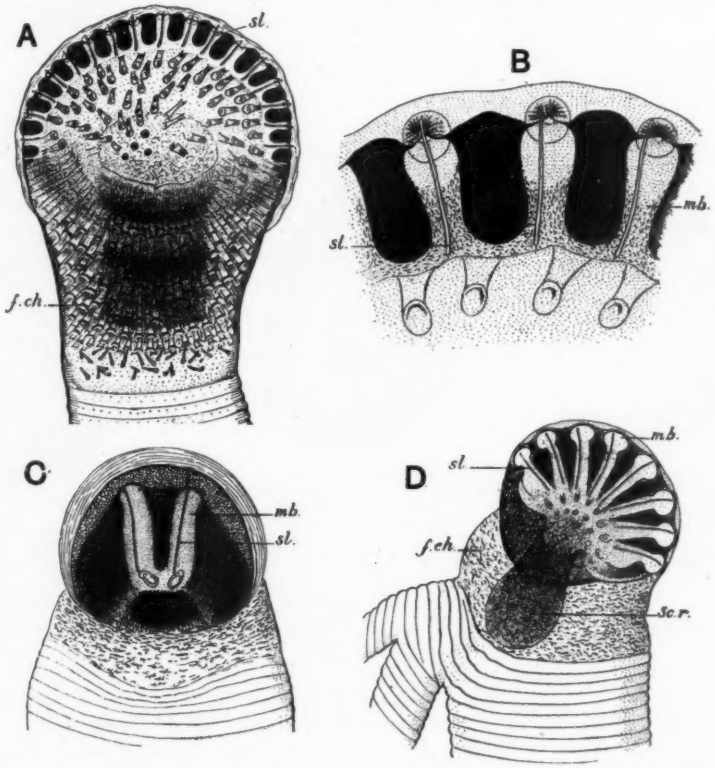


Fig. 7. *Laphria* sp.: A, prothoracic spiracle of the larva and B, detailed structure of its peripheral region. *Thereva* sp.: C, prothoracic and D, postabdominal spiracles of larva. *f.ch.* felt chamber; *mb.* membrane; *sc.r.* scar rod; *sl.* slit. A × 180, B × 600, C and D × 300.

The cord appears, however, to penetrate into the spiracular chamber (D) in a manner similar to the scar plugs of the second type of spiracles previously described. The thin chitinous membranes which cover each of the papillae show very narrow slits (*sl.*) which can easily be seen on transverse sections (Fig. 10 A, C) and sections parallel to the surface of the plate (Fig. 10 D–I).

The papillae are accompanied by the very small openings (*g.o.*) of a perispiracular gland, and in papillae (A, *sp.p.*) are very numerous and show a distinct longitudinal slit (C, F, *sl.*) in their thin membrane (*mb.*).

The moult which separates the second and third larval stages (Fig. 11 A) shows that the old spiracular plate (*sp.pl.II*) is completely enclosed within the new plate (*sp.pl.III*), so that the external scar (B, *e.sc.*) which is formed after removal of the old tracheo-spiracular system lies within the new plate slightly nearer to its internal border; the ecdysial

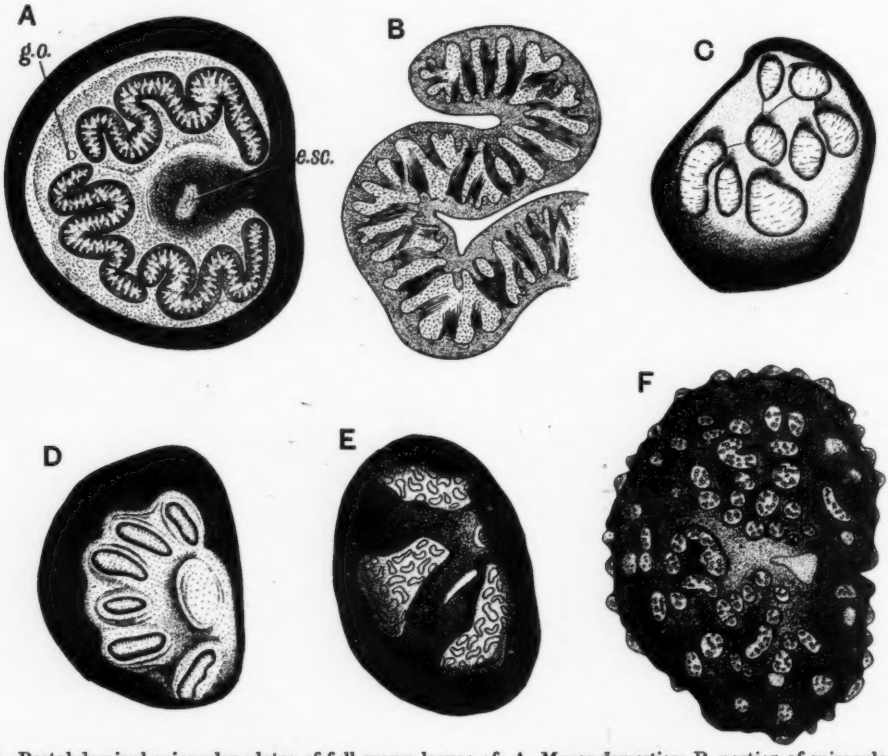


Fig. 8. Postabdominal spiracular plates of full-grown larvae of: A, *Musca domestica*; B, portion of spiracular pertreme of this larva; C, undetermined larva parasitic in *Lithobius* sp.; D, *Viviania cinerea* Fall.; E, *Carcelia gnava* B. & B.; F, *Pelatachina tibialis* Fall. Figs. C, D and E after Nielsen (1909), Fig. F after Nielsen (1916). A ×240, B ×800, C ×430, D ×85, E ×65, F ×95.

section they show free spaces or lacunae (*lac.*) connected with the finger-like processes of the felt chamber (*f.*) filled in places with a very dense chitinous felt (Fig. 10 B). The latter gradually gives way to large open spaces of the spiracular chamber (*Sp.ch.*) with a layer of felt bordering only its walls. The basal portion of this chamber is followed by a well-developed tracheal trunk.

(6) *Oestrus ovis* L.

The postabdominal spiracles of this larva (Fig. 11) resemble those of *Oedomagena tarandi*. The spiracular tube in such a position, after complete contraction and obliteration of its lumen, will form a structure similar to a scar plug of the second type of spiracles.

It is interesting to note that the external scar of the second stage larva (Fig. 11 D, *e.sc.*) lies much nearer the internal border of the spiracular plate. The spiracular plate of the second stage liberated after the moult shows 20–25 tufts of very fine chitinous strands arising from its inner surface (D, *ch.str.*). Unfortunately the lack of fresh material made it impossible to ascertain the nature of these strands.

(7) *Structural variations of spiracles in other larvae*

The few examples which have been examined above are far from exhausting the list of variations which may be exhibited by the larvae of Diptera. All these variations may be summarized as follows:

(i) The position of the external scar in relation to spiracular clefts may show important variations. As was shown before, the scar may lie in the centre of the spiracular plate being then surrounded by spiracular clefts, or at its periphery or even lie completely outside the spiracular plate. In postabdominal spiracles, if the scar does not lie in the centre of the plate, it is always found near its internal border. On other segments the scar is found either in front of, or behind the scar (Fig. 20 B, C), and

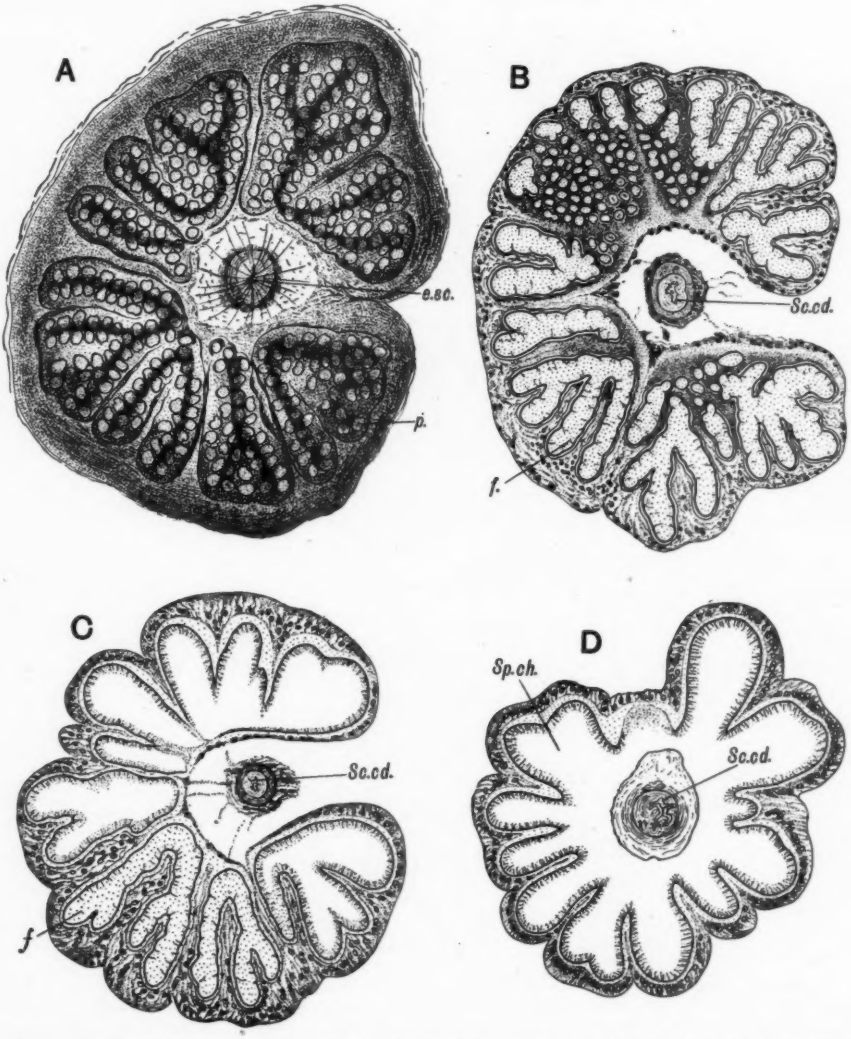


Fig. 9. *Oedomagena tarandi* (L.), postabdominal spiracle of a full-grown larva. A, left spiracular plate; B, C and D, sections at successive depths of spiracle showing the transition between felt and open spiracular chambers leading towards tracheal trunk. *e.sc.* external scar; *f.* felt; *p.* spiracular papillae; *Sc.cd.* scar chord; *Sp.ch.* spiracular chamber. A–D × 65.

this, as we shall see (p. 25), is a very important point to remember.

The scar is usually simple; in some cases, however, it may be double (postabdominal spiracle of *Bibio*

(Fig. 46, p. 55). The terminal portion of the felt chamber, especially in the prothoracic spiracles of cyclorrhaphous Diptera, may protrude from the surface of the segment and expand, giving rise to a

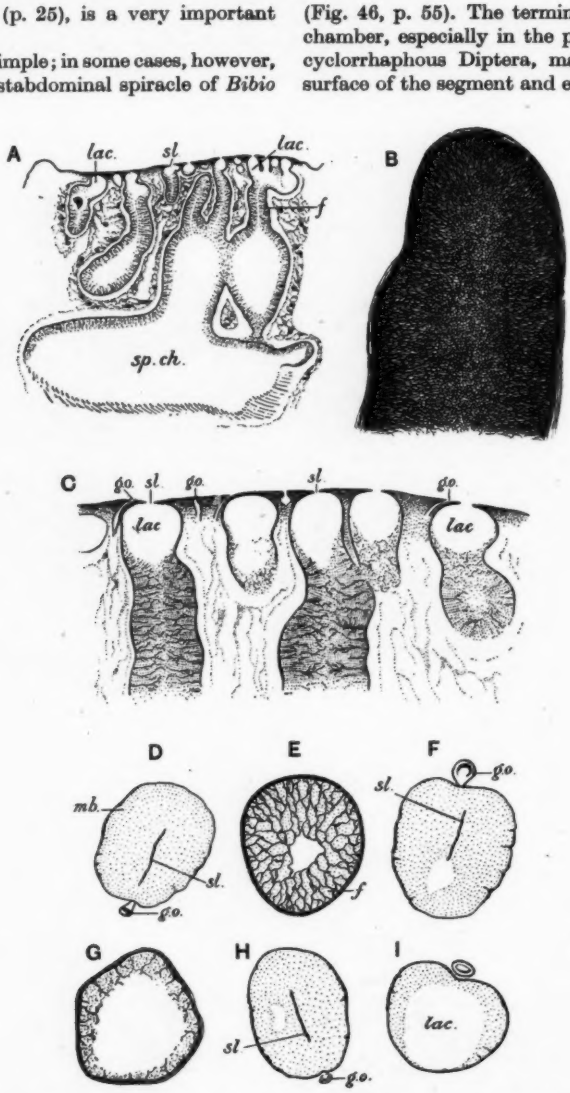


Fig. 10. *Oedomagena tarandi* (L.) postabdominal spiracle of a full-grown larva. A, vertical section; B, section of chitinous felt; C, vertical section of papillae showing more details than A; D–I, spiracular papillae surface view and sections parallel to the surface. *f.* chitinous felt; *g.o.* external opening of perispiracular gland; *mb.* thin chitinous membrane covering the papillae; *lac.* lacunae devoid of felt; *sl.* slit in membranes of the papillae; *sp.ch.* spiracular chamber. A × 65, B × 600, C × 300, D–I × 600.

larvae) or even triple (postabdominal spiracle of the larva of *Dilophus*).

(ii) The spiracle may either form a flat disk on the surface of the segment or may project above the surface as is the case in the larvae of *Glossina palpalis*

fringe of spiracular papillae (ex. *Acidia heraclei*, Fig. 12 A, p. 14).

(iii) The papillae may form long finger-like projections arising at the same level of the felt chamber (prothoracic spiracles of *Drosophila*) or in pairs all

along a single projection of a felt chamber (*Aulacogaster rufitarsis*, Fig. 51, p. 59).

(iv) The spiracles are usually exposed on the surface of the segment. In certain species, however, they are enclosed in a deep furrow of the segment, as is the case with the prothoracic spiracles of *Drosophila*, *Aulacogaster*, *Gastrophilus* (Fig. 13 A, B) and *Dermatobia* (Fig. 42 A–C). Whereas in the first two cases the spiracles may be seen occasionally evaginated in *Gastrophilus* and *Dermatobia* the evagination takes place only during formation of the puparium (Fig. 42 D, p. 50).

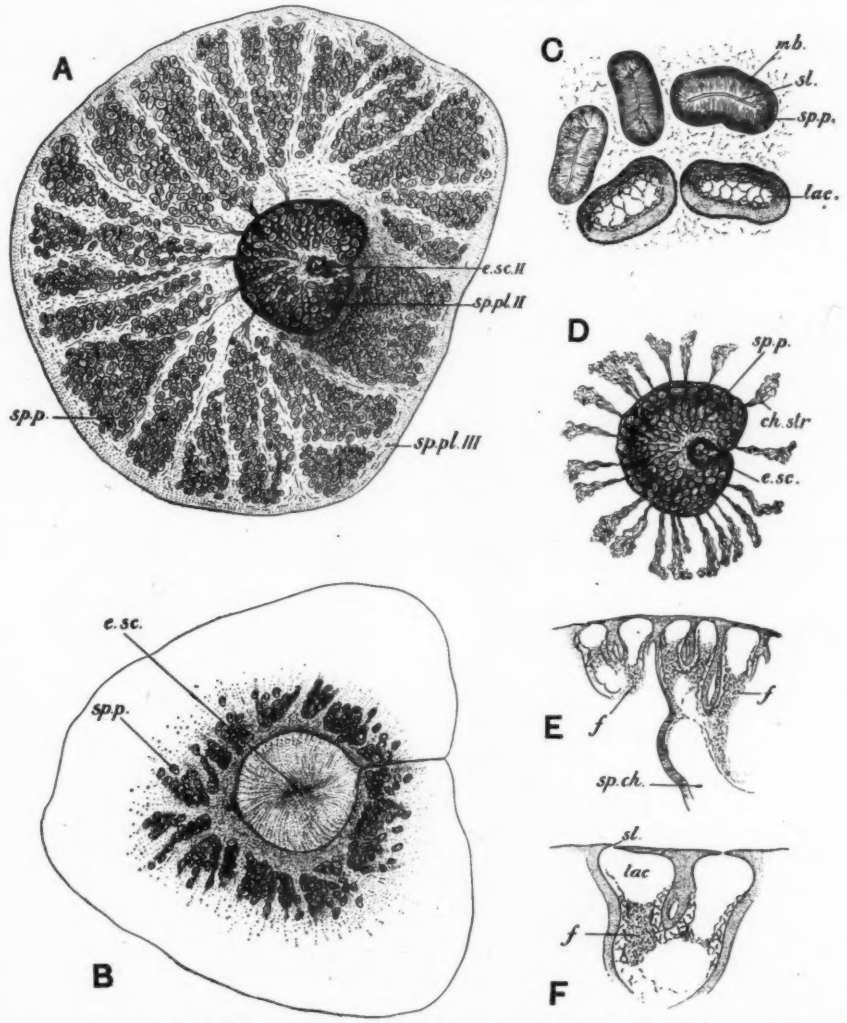


Fig. 11. *Oestrus ovis*, postabdominal spiracle of larva. A, left spiracular plate during moult separating second and third larval stages; B, similar spiracular plate after moult showing contraction of external scar after removal of tracheo-spiracular system of st. II. Only spiracular papillae surrounding the external scar are represented in this figure; C, surface views of spiracular papillae at different levels of focusing; D, spiracular plate of st. II expelled from st. III; E, vertical section of spiracular plate st. III; *F*, as E, but more highly magnified. *ch.str.* chitinous strands of undetermined nature; *e.sc.* external scar; *e.sc. II*, external scar of spiracular plate st. II; *f.* felt chamber; *lac.* lacunae; *mb.* membrane of spiracular papillae; *sl.* slit in this membrane; *sp.p.* spiracular papillae; *sp.pl. II*, and *sp.pl. III*, spiracular plates of second and third larval stages. A, B and D × 65, C and F × 600, E × 300.

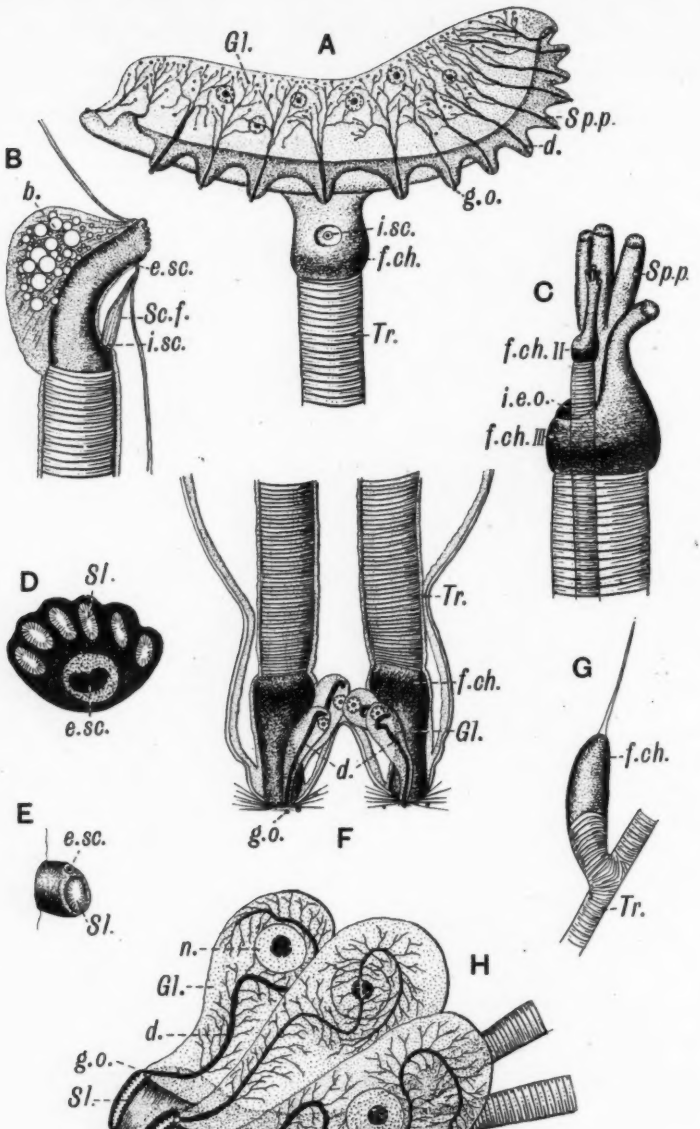


Fig. 12. Spiracles and perispiracular glands in dipterous larvae. A, *Acidia heraclei* L., prothoracic spiracle and perispiracular gland; B, *Mycetobia pallipes* Meig., prothoracic spiracle and perispiracular gland; C, *Pollenia rudis* Fab., moulting of prothoracic spiracles separating second and third larval stages; D, E, *Mycetophila punctata* Meig., prothoracic and abdominal spiracles; F, *Paraspiniphora bergenstammi* Mik., postabdominal spiracles and perispiracular glands; G, *Hydrellia modesta* L., prothoracic non-functional spiracle (see p. 29); H, *Acidia heraclei*, postabdominal spiracle and perispiracular glandular cells. *b.* globules of secretion; *d.* glandular ducts; *e.sc.* external scar; *f.ch.* felt chamber; *Gl.* perispiracular glands; *g.o.* their external openings; *i.e.o.* internal ecdysial opening; *i.sc.* internal scar; *n.* nuclei of perispiracular glandular cells; *Sc.f.* scar filament; *Sl.* spiracular slits; *Sp.p.* spiracular papillae; *Tr.* tracheal trunk. A ×240, B ×330, C–F ×170, G ×260, H ×240.

(v) The postabdominal spiracles in calliphorines and more specially in sarcophagines are found in a more or less deep cup-like depression which by the play of muscles may either expose or protect the spiracles. In larvae of *Gastrophilus* the postabdominal spiracles lie in a deep fold, the lips of which are either widely open or completely and almost hermetically closed over the spiracular plates (Fig. 43, p. 52).

In larvae of certain Stratiomyidae the postabdominal spiracles are found within a deeply and permanently invaginated sac communicating with the outside by means of a narrow slit (Fig. 33, p. 40).

(vi) In other species the postabdominal spiracles are supported by more or less long projections of the last abdominal segments. In *Melanochelia riparia* (an anthomyid larva) (Fig. 28 A, p. 36) there are two of these projections, each supporting one spiracle. In larvae of *Ephydra riparia* a single projection bifurcates terminally, each branch carrying a spiracle (Fig. 28 B). In other forms a single projection supports both spiracles lying close together. Such a projection, described as a siphon, may be of different length. It is very common in mosquito larvae (Fig. 29, p. 36), in larvae of aquatic stratiomyids, in *Ptychoptera* and several eristalines.

IV. PERISPIRACULAR GLANDS

Leydig (1859) was the first to find unicellular hypodermic glands connected with spiracles in insects.

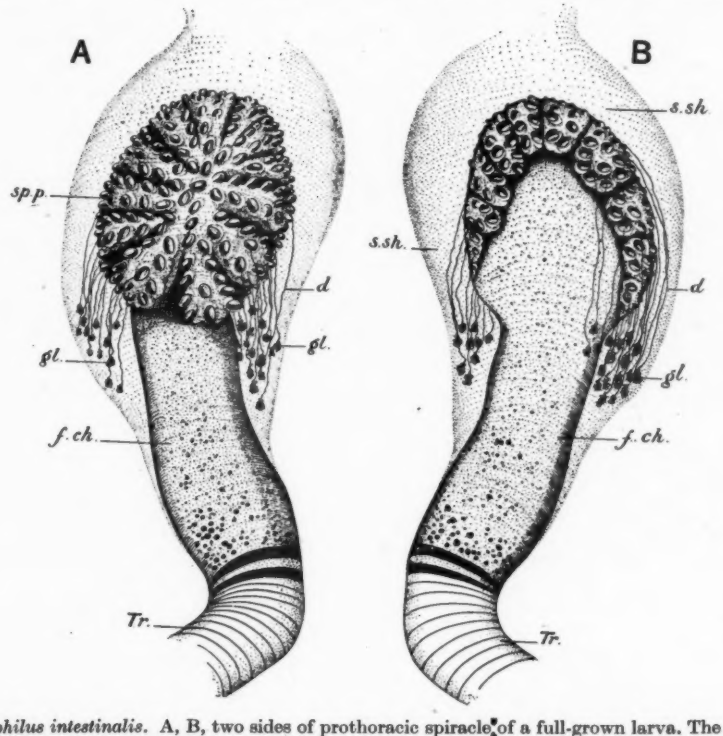


Fig. 13. *Gastrophilus intestinalis*. A, B, two sides of prothoracic spiracle of a full-grown larva. The glandular mass is omitted in this figure in order to show the distribution of individual ducts and their intracellular coils. *d.* ducts of perispiracular gland; *f.ch.* felt chamber; *gl.* coils of intracellular glandular ducts; *sp.p.* spiracular papillae; *s.sh.* spiracular sheath; *Tr.* tracheal trunk. A and B × 65.

In 1879 Batelli described in the larva of *Eristalis* a group of cells connected with the spiracles and compared them with unicellular glands found by Leydig in other insects. Viallanes (1885) independently described these cells in the same larva, and although his description was correct in every respect, he completely misunderstood the nature of these cells, ascribing to them the function of a special elastic organ serving for protrusion and the retraction of the respiratory siphon of *Eristalis* larvae. Gazagnaire (1886), without making a personal study of these cells and relying only upon Viallanes's

descriptions and figures, drew the right conclusion as to their nature and function. The long refringent sinuous filament rolled up within the protoplasm of these cells and mistaken by Viallanes for an elastic cord was rightly understood by Gazagnaire to be a simple intraprotoplasmic duct filled with an oily secretion which serves to lubricate the portion of the spiracle surrounding the external opening. Wahl (1899) confirmed Gazagnaire's interpretation and found that the intraprotoplasmic ducts open in the spiracular plate. The oily secretion which exudes from the duct covers the 'rosette' of long chitinous hairs which surround the spiracles, thus setting up a negative meniscus on the surface film of the water. This enables the *Eristalis* larvae to remain suspended in the fluid medium while maintaining their spiracles in communication with the free air. Pantel (1901), who found similar cells in several parasitic and free-living larvae, accords them the same structure and function as did Gazagnaire and Wahl.

I described these cells in the larvae of *Phora* and *Trichocera*, and (Keilin, 1913) showed that these glands are present in all dipterous larvae, being always connected with functional spiracles. In the same paper I also called attention to the striking resemblance of these glands to hypodermal gland cells of several tipulid larvae (*Ula*, *Dicranomyia*, *Limnophila*, *Mongoma* and *Epiphragma*). The presence and nature of these glands in dipterous larvae were confirmed by Krüger (1926) in *Syritta*, by Dolley and Farris (1929) in different stages of *Eristalis* larvae, by Bates (1934) in larvae of certain trypetids, by Butt (1937) in a larva of an undetermined trypetid, and by Phillips (1939) in the larvae of *Rhagoletis suavis*.

The structure of the perispiracular or peristigmatic glands can be best seen by direct microscopic examination of transparent living larvae compressed between a slide and cover-slip. Their structure varies according to the species and the position of the spiracle. The following description and figures of a few examples of these glands will give us a general idea of their structure and variations.

Acidia heraclei L. The perispiracular glands in the celery-fly larvae have been described and figured by Keilin & Tate (1943). The glands connected with post-abdominal spiracles (Fig. 12 H) consist of three large pyriform cells each showing a large nucleus (*n.*) and a long sinuous intraprotoplasmic duct (*d.*) filled with a refringent liquid and giving off a large number of secondary ramified ductules extending towards the periphery of the cell, where they terminate in strings of refringent oily droplets. The duct of each cell opens at the external margin of the peritreme surrounding each of the three spiracular clefts (*g.o.*). The secretion of these glands is distributed over the peritreme of the spiracles and the hairs which surround them.

The prothoracic perispiracular glands (Fig. 12 A) form a compact multicellular mass (*Gl.*) traversed by several ramified ducts (*d.*) which open near the extremity of each of the fifteen papillae of a transversely expanded felt chamber of the spiracle. The ducts and their ramifications are filled with the refringent secretion.

Paraspiniphora bergenstammi Mik. The post-abdominal perispiracular gland of this larva (Fig. 12 F) is composed of two large cells which lie alongside the felt chamber. The intraprotoplasmic duct is curved at its internal extremity, near the nucleus of the cell, and opens in the peritreme surrounding the spiracular clefts.

Mycetobia pallipes Meigen. The prothoracic gland of this larva (Fig. 12 B) forms a single mass of fused cells filled with several strings of globules of different sizes connected by very narrow hardly perceptible protoplasmic clefts representing the ducts. A similar structure is found in the gland of the prothoracic spiracle in the larva of *Trichocera*.

Gastrophilus intestinalis. The glands of the prothoracic spiracle of this larva (Fig. 13 A, B) form a thick cellular mass surrounding the felt chamber. Each of the numerous spiracular papillae is connected with a long intraprotoplasmic glandular duct terminating in a compact coil lying deep within the glandular mass at a considerable distance from the corresponding papilla. In this respect the structure of the gland resembles that of *Eristalis tenax*.

The larvae of *Culex pipiens* L. and *Anopheles claviger* Mg. The perispiracular glands in mosquito larvae were recently described and figured (Keilin, Tate & Vincent, 1935). They can be seen easily in freshly moulted 4th stage larvae of *Culex pipiens* where they form a multicellular mass surrounding the felt chamber of the postabdominal spiracle. The cells show numerous refractile globules of oily secretion staining black with osmic reagents and red with Sudan III. The globules are connected by hardly perceptible protoplasmic clefts representing the ducts.

The study of these glands in larvae of many other species of Diptera shows that they differ very little from one or the other form described above. There is little doubt that the function of these glands consists in secreting a hydrofuge substance which, by covering the spiracles and the perispiracular region, protects them from being wetted and enables aquatic larvae like those of *Eristalis* and mosquitoes to bring their spiracles into contact with the atmospheric air.

V. CLASSIFICATION OF THE RESPIRATORY SYSTEMS IN DIPTEROUS LARVAE

It is now generally accepted that the highest number of functional spiracles in existing insects is ten pairs, two of which are thoracic and eight abdominal. The first classification of respiratory systems in insects, based upon the number and distribution of spiracles, we owe to Schiner (1862) and to Brauer (1869) who distinguished only three systems: metapneustic, amphipneustic and peripneustic. Subsequently this classification was revised and completed by Palmén (1877) in his classical paper on the tracheal system of insects. This paper still remains one of the fundamental contributions to the study of this subject, and I shall have to refer to it on several occasions. Palmén divided the respiratory systems into three large groups: (1) apneustic, (2) hemipneustic and (3) holopneustic, subdividing then the hemipneustic into (*a*) metapneustic, (*b*) amphipneustic, (*c*) propneustic, (*d*) peripneustic and (*e*) hemipneustic mixed. Palmén's classification was accepted with some minor modifications by Steinke (1919), de Gryse (1926) and other workers.

In the course of the present study, Palmén's main observations were confirmed and certain of his generalizations were accepted, but the great variety of examples investigated made it necessary to modify his classification of the respiratory systems. These modifications are all the more important since Palmén often used the same term for different systems and vice versa.

The proposed classification of the respiratory systems, based on the study of a large variety of dipterous larvae, can be summarized as follows:*

1. *Holopneustic:* 10 pairs of spiracles; 1 prothoracic, 1 metathoracic and 8 abdominal. Examples: larvae of Bibionidae (*Bibio*, *Dilophus* and *Plecia*).

2. *Peripneustic:* 9 pairs of spiracles; 1 prothoracic and 8 abdominal. Example: larvae of Scatopsidae, Ditomyidae, Cecidomyidae.

3. *Hemipneustic:* 8 pairs of spiracles; 1 prothoracic and 7 abdominal. Example: almost all Mycetophilidae larvae; the aberrant larva of a Cecidomyidae (*Miastor*) will be considered separately.

4. *Amphipneustic:* 2 pairs of spiracles; 1 prothoracic and 1 postabdominal. Examples: larvae of Trichoceridae, Anisopodidae, Psychodidae, Thaumaleidae, Dixidae, Stratiomyidae, Xylophagidae, Dolichopodidae, Empididae, etc., and the majority of the larvae of cyclorrhaphous Diptera.

5. *Metapneustic:* 1 pair of spiracles, the postabdominal pair. Examples: Tipulidae, Culicidae, several forms of Diptera Brachycera.

6. *Propneustic:* 1 pair of spiracles; the prothoracic pair. Examples: larvae of a few mycetophilids such as *Speolepta* and *Diodocidia*. System common in pupal stage.

7. *Apneustic:* No functional spiracles. Examples: larvae of Chironomidae, Blepharoceridae, Simulidae, *Chaoborus* (Culicidae), *Ceroplatus* (Mycetophilidae), *Antocha* (Tipulidae).

It must be remembered that not all spiracles of the larva are of the same size and the same complexity of structure. In holopneustic and peripneustic systems the prothoracic and postabdominal spiracles and in hemipneustic the prothoracic spiracles only are more developed than the other spiracles of the body. In all forms except hemipneustic and propneustic the postabdominal spiracles are the most developed. Finally, the prothoracic spiracles differ from the postabdominal spiracles not only in structure but probably also in function.

VI. FUNCTIONAL AND NON-FUNCTIONAL SPIRACLES

De Geer (1771) was the first to find that the cast-off cuticle of a moulted aquatic larva of *Libellula* contains all the tracheal tubes expelled from the body.

In 1841 Pictet discovered the existence of unperforated spiracles in the larvae of Perlidae, and Oustalet (1869), who found them in Libellulidae, raised the question as to their significance in aquatic larvae and pupae.*

Although the moulting of the tracheal system in the apneustic aquatic larvae as well as the presence of the closed rudimentary spiracles were noticed by several workers, neither the mechanism of such a moulting process nor the significance of these spiracles were properly understood.

The solution of these problems, which we owe to Palmén (1877), was an achievement of great merit and forms an outstanding contribution to the knowledge of the tracheal system of insects. It deserves therefore to be carefully examined. According to Palmén the respiratory system of ephemerid larvae is completely closed. The two tracheal trunks, instead of sending off in each segment of the body a pair of branches terminating in spiracles, are connected with the body wall of each segment (from 2nd to 11th) by means of ten pairs of thin solid cords. Each cord is composed of a central chitinous layer connecting the intima of the tracheal trunks with the

* Only the full-grown larvae are considered in this classification.

* Oustalet (1869, p. 385) asks: 'Ceux-ci sont-ils seulement des témoins qu'occuperont les orifices respiratoires de l'adulte, ou bien servent-ils déjà à la nymphe pour l'absorption des gaz, lorsqu'elle est obligée de respirer à l'air-libre, soit par suite du desséchement de la mare, qu'elle habite, soit surtout quand arrive l'instant de sa métamorphose et quand ses branchies commencent à s'atrophier?'

cuticle of the body wall and a peripheral, epithelial layer forming a link between the hypoderm of the skin and the flattened epithelium of the tracheal trunks. During the moult the epithelial layer of the cord becomes detached from the chitinous filament, grows and secretes a chitinous tube surrounding the filament and uniting the new tracheal tube to the newly formed cuticle of the body. The old tracheae break up into several portions which, being attached by the filaments to the old cuticle, are eliminated from the larval body through the newly secreted tubes. Once the moulting is completed these tubes collapse, their lumen disappears and they are transformed into solid chitinous cords. The moult which separates the sub-imago from the pupa proceeds in a similar manner except that the tubes do not close after the moulting but remain open, giving rise to functional spiracles and their tracheal branches.

The number of functional spiracles in the sub-imago and the imago is ten pairs, two thoracic and eight abdominal, and this is exactly the number of non-functional spiracles and their closed branches or cords found in the larva and pupa of these insects. Similar results were obtained by Palmén from the study of aquatic larvae of other insects such as Perlidae, Libellulidae, Sialidae, Trichopteridae and Corethra among the Diptera.

Since Palmén's work very little attention has been paid to this subject, and even in the papers dealing specifically with the tracheal system of insects, the non-functional spiracles and their obliterated branches were seldom mentioned. Among dipterous larvae the spiracular filaments were mentioned by Pratt (1893) in the larvae of *Melophagus ovinus*, by Wahl (1899) in that of *Eristalis tenax*, by Pantel (1901) in certain larvae parasitic in insects, by Imms (1907) in larvae of *Anopheles maculipennis*, by Carpenter & Pollard (1918) in the larvae of *Hypoderma* and *Oedomagena* and by Dunavan (1929) in larvae of *Eristalis tenax*.

In the course of the present investigation the non-functional spiracles and the chitinous filaments connected with them were found in the larvae of a great number of Diptera belonging to the following families: Mycetophilidae, Ditomyidae, Scatopsidae, Cecidomyidae, Tipulidae, Anisopodidae (= Rhyphidae), Simulidae, Chironomidae, Culicidae, Stratiomyidae, Leptidae, Dolichopodidae, Syrphidae, Phoridae, Anthomyidae, Drosophilidae, Agromyzidae, Ephydridae, Metopiidae.

The non-functional spiracles are present in every dipterous larva which has less than ten pairs of functional spiracles bringing up its total number of spiracles to ten pairs. The respiratory systems previously examined can now be easily represented schematically (Fig. 14 and Table 1) by means of simple formulae, taking into consideration both the functional (S) and non-functional (N) spiracles of the three thoracic (*I*, *II* and *III*) and eight abdominal (1–8) segments (Table 1).

Table 1

Respiratory systems	Spiracular formulae	$S+N$	Total
Holopneustic	$S_I S_{III} S_{1-8}$	$10S+0N$	10
Peripneustic	$S_I N_{III} S_{1-8}$	$9S+1N$	10
Hemipneustic	$S_I N_{III} S_{1-7} N_8$	$8S+2N$	10
Amphipneustic	$S_I N_{III} N_{1-7} S_8$	$2S+8N$	10
Metapneustic	$N_{I,III} N_{1-7} S_8$	$1S+9N$	10
Propneustic	$S_I N_{III} N_{1-8}$	$1S+9N$	10
Apneustic	$N_{I,III} N_{1-8}$	$0S+10N$	10

The study of a large number of dipterous larvae which is summarized in Figs. 14 and 15 and Table 1, leads to the following generalizations:

(1) The total number of spiracles: functional (S) and non-functional (N) in all the respiratory systems is ten pairs.

(2) The holopneustic system can be considered as the most complete and perhaps the most primitive, having all spiracles functional.

(3) In respect to the number of functional spiracles, the respiratory systems of dipterous larvae can be separated into three main groups: (*a*) polypneustic with at least eight pairs of spiracles; (*b*) oligopneustic with one or two pairs of spiracles; (*c*) apneustic with no functional spiracles.

(4) There are no transitional forms between the poly- and oligopneustic respiratory systems.

(5) The mere fact of the existence of non-functional spiracles and their branches gives no information as to the phylogenetic position of the insect in question.

(6) There are no spiracles, functional or non-functional, on the mesothoracic segment.

The last three generalizations deserve special consideration and will be examined in some detail.

VII. ABSENCE OF TRANSITIONAL FORMS BETWEEN POLYPNEUSTIC AND OLIGOPNEUSTIC SYSTEMS

The lack of intermediate forms between the poly- and oligopneustic systems is due to the fact that the first seven pairs of abdominal spiracles are either all present or all absent. Palmén (1877, pp. 83–5) believed, however, that the amphipneustic muscid larvae may bear three or four pairs of spiracles. This view was based upon Weissmann's observation (1864) that the postabdominal spiracles of the second and third larval stages in blowflies show two and three spiracular clefts respectively. According to Palmén the 6th and 7th abdominal spiracles which are non-functional in the first larval stage, during the successive moults, instead of opening on the segments to which they belong, migrate upon the

Segments	I	II	III	1	2	3	4	5	6	7	8	
1 Holopn. pr.	O	O	O	O	O	O	O	O	O	O		
2 Holopn.	O		O	O	O	O	O	O	O	O	O	Polypneustic
3 Peripn.	O		•	O	O	O	O	O	O	O	O	
4 Hemipn.	O		•	O	O	O	O	O	O	O	•	
5 Amphipn.	O		•	•	•	•	•	•	•	•	O	Oligopn.
6 Metapn.	•		•	•	•	•	•	•	•	•	O	
7 Propn.	O		•	•	•	•	•	•	•	•	•	
8 Apn.	•		•	•	•	•	•	•	•	•	•	Apn

O funct. S • non funct. N

Fig. 14. Schematic representation of respiratory systems in dipterous larvae. *I*, *II* and *III*, three thoracic segments: pro-, meso- and metathorax; 1–8, eight abdominal segments; *N*, non-functional spiracles with obliterated spiracular branches; *S*, functional spiracles; *Holopn. pr.*—primitive holopneustic system.

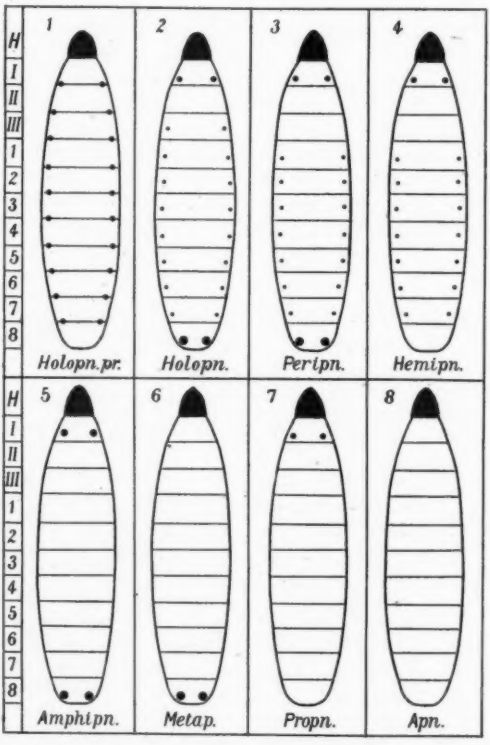


Fig. 15. Schematic representation of respiratory systems in dipterous larvae showing only functional spiracles. *I–III* and 1–8 as in Fig. 14; *H*, head

last abdominal segment and open near the 8th pair of spiracles, thus forming a compound spiracle with two or three clefts.

A similar explanation as to the structure of post-abdominal spiracles is found in the papers by Pratt (1893, pp. 179–80) on *Melophagus ovinus* and by Carpenter & Pollard (1918, p. 76) on *Hypoderma*. This view is also accepted in the classification of respiratory systems proposed by de Gryse (1926). The incorrectness of such a view has been shown already by Pantel (1901) in his interesting note on the rudimentary organs of muscid larvae.

The study of amphipneustic larvae of orthorrhapous and cyclorrhapous Diptera during the successive stages of their development clearly shows that all the non-functional spiracles (one metathoracic and seven abdominal) remain in their corresponding segments, while the last abdominal spiracle acquires two or three clefts or becomes modified in some other way. The simultaneous closing of eight pairs of spiracles (one metathoracic and eight abdominal) is probably due to the partial submersion of the larva in a liquid medium, a factor which acts more or less uniformly on all segments of the body except the two terminal ones, which are in a better position to bring their spiracles in contact with the air.

The development and specialization of the post-abdominal spiracles take place without the migration of any spiracles belonging to other segments. In fact even in holopneustic larvae like those of *Bibio*, *Dilophilus* and *Plecia* the postabdominal spiracles are highly developed and have the appearance of being double or triple, while the spiracles on all other abdominal segments remain functional.

VIII. PHYLOGENETIC SIGNIFICANCE OF NON-FUNCTIONAL SPIRACLES

This problem was opened by Palmén (1877) who, after careful study of non-functional spiracles in aquatic larvae, concluded that it was inconceivable that they were rudimentary from the beginning and originated phylogenetically as filaments. On the contrary, the larvae which at present have a closed tracheal system had previously open spiracular branches in all the stages of their life. Palmén summarizes this view in a clear statement that 'from a *relatively primitive*, open form of tracheal system of ancient insects originated phylogenetically, the *relatively secondary*, closed form of the present time larvae'. This statement has been amply confirmed by the results of the present investigation.

However, not being aware of Palmén's important work, Carpenter & Pollard (1918) approached this problem in their study of *Hypoderma*. In a full-grown larva they found only six pairs of non-functional spiracles belonging to 2–7 abdominal segments; they failed to find these spiracles in the earlier larval stage and they could not detect them in the larvae of other flies such as the blue-bottle and *Tipula* sp. These observations led them to the following conclusion: 'If these lateral spiracles are indeed absent from many genera of muscoid flies in the larval stage, their presence must be regarded as a definitely primitive character, and it becomes all the more surprising to find them in the maggot like that of *Hypoderma*, specialized in many ways for a parasite life and remarkable for the extreme reduction of the anterior region, so that the front spiracles, belonging to the thorax, lie only just behind the mouth. The facts set forth in this paper suggest that, if *Hypoderma* be really near any of the typical muscoids, it must be diverged from the common stock before these larval lateral spiracles had been lost, so that the specialized parasitic habit of the group must have begun at a period comparatively remote from to-day.'

All this differs markedly from the results obtained in the course of the present study. In fact, the amphipneustic larvae of the blue-bottle and the metapneustic larvae of *Tipula* have their usual number of non-functional spiracles—eight pairs in the first instance and nine pairs in the second. It is most probable that the larvae of *Hypoderma* have also eight pairs of non-functional spiracles and not six as described by these authors. Carpenter & Pollard were misled by the fact that, owing to some peculiarities in the texture of the larval cuticle in *Hypoderma*, the non-functional spiracles, which are nothing but scars left after contraction of ecdysial openings, are somewhat more strongly sclerotized. They have naturally looked for similar structure in other larvae, and having failed to find them, ascribed to their presence in *Hypoderma* a special phylogenetic significance, which they do not possess.

IX. FATE OF THORACIC SPIRACLES IN LARVAE

The absence of a few functional spiracles does not necessarily imply the adaptation of the larva to partly submerged conditions of life. In fact, the larvae of the great majority of holometabolic insects are devoid of the second and third pairs of functional spiracles notwithstanding that they have a terrestrial mode of life and possess nine pairs of functional spiracles (one prothoracic and eight abdominal). There are, however, exceptions to this rule; thus the larvae of Bibionidae have ten pairs of functional spiracles (one prothoracic, one metathoracic and eight abdominal), the mesothoracic segment only being devoid of spiracles. What is still

more important is that the absence of functional spiracles has not always the same significance, especially if it concerns the mesothoracic and the metathoracic segments.

According to Palmén (1877) the total number of spiracles (functional and non-functional) in larvae which have functional prothoracic spiracles is eleven pairs. This applies to all larvae whether their respiratory system is peripneustic, hemipneustic, amphipneustic or propneustic. In all these cases Palmén describes and represents schematically three thoracic and eight abdominal pairs of spiracles. Of the three thoracic pairs he finds one functional and two non-functional.

The study of thoracic segments in a large number of dipterous larvae shows, however, that this view is incorrect. The three thoracic segments in all larvae of Diptera and Aphaniptera have only two pairs of functional or non-functional spiracles, one pair on the prothoracic and one on the metathoracic segment, the mesothoracic segment being completely devoid of spiracles. The absence of a functional spiracle on a segment may therefore mean either the presence of a non-functional spiracle or a complete absence of all traces of spiracles. The question that now arises is how to explain a total absence of spiracles on a segment which is intercalated between two other segments possessing functional or non-functional spiracles.

The answer to this question will be found in the study of the metamerism of the tracheo-spiracular system.

X. METAMERISM OF TRACHEO-SPIRACULAR SYSTEMS

Of all the organs of insects the tracheal system shows undoubtedly the most perfect metameric structure. This metamerism is specially striking in the early stages of development of the tracheal system which is formed by a series of lateral paired invaginations of integument giving rise in each segment to a pair of lateral bundles of more or less developed tracheae. The unspecialized form of tracheal system, in which the tracheal bundles of each segment retain their complete independence, is met with only in a few primitive insects such as *Machilis* (Thysanura). The tracheal system in other insects, and especially in dipterous larvae, is, on the contrary, united into one highly specialized system composed usually of two pairs of longitudinal tubes running throughout the length of the body and giving rise in each segment to spiracular branches and branches ramifying in different organs of the larva. This complicated tracheo-spiracular system can be divided into a series of similar tracheal complexes corresponding to each segment of the larval body. However, such complexes, in spite of their regular recurrence, do not correspond with the real metameres.

To detect the latter it does not suffice to divide the tracheal system into more or less similar portions strictly corresponding to each segment of the body. True metameres must represent the original independent units which by joining together form the more specialized tracheal system. A careful study of the tracheal system in a dipterous larva reveals that the spiral thickening of the cuticular intima is not always continuous, being interrupted in certain places by very short rings of modified texture and contours. These structural interruptions of the tracheae mark undoubtedly the places of junction of tracheal tubes belonging to independent units. These places of junction enable us to divide the tracheal system into a series of tracheal branches arising from a corresponding spiracle and forming real tracheo-spiracular metameres.

We shall now examine in some detail the metameric structure of hemipneustic, holopneustic, peripneustic, amphipneustic and apneustic respiratory systems. In the descriptions which follow only the main tracheal branches will be mentioned.

(a) *Tracheo-spiracular metameres of hemipneustic system*

The living transparent larvae of *Mycetophila cingulum* Meigen, compressed between a slide and a cover-slip and examined from the side, are exceptionally suitable for this kind of study. To facilitate the interpretation of the structures thus observed the tracheae belonging to adjacent metameres are represented in two different shadings: uniformly black or transversely striated.

A glance at Fig. 16 shows that the prothoracic functional spiracle S_I gives off two main tracheal branches directed posteriorly: its portion of tracheal trunk (*Tr.I*) and a posterior-ventral branch (*tp.I*). These two main branches of the 1st metamere meet and join, at points *n* and *m*, the corresponding branches *Tr.III* and *t.a.III* originating from the non-functional spiracle N_{III} of the metathoracic segment. All tracheae and their branches which are found in the mesothoracic segment derive from the prothoracic (S_I) and metathoracic (N_{III}) metameres. It is interesting to note that the tracheae of each segment, except the two terminal ones, belong to two metameres. For instance, the tracheal system of the metathoracic segment derives from its own metamere (N_{III}) and that of the first abdominal segment (S_1) and so on. The tracheae of the last abdominal segment (Fig. 17 C) belong, however, only to one metamere arising from the last non-functional spiracle (N_8). This metamere supplies also two important tracheal branches *Tr.*8 and *t.a.*8 to the 7th abdominal segment.

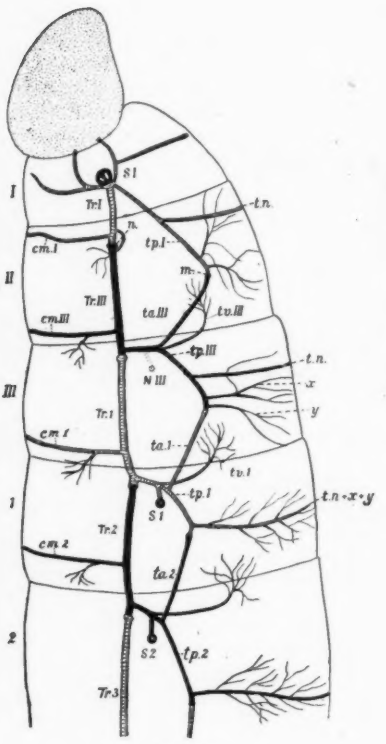


Fig. 16.

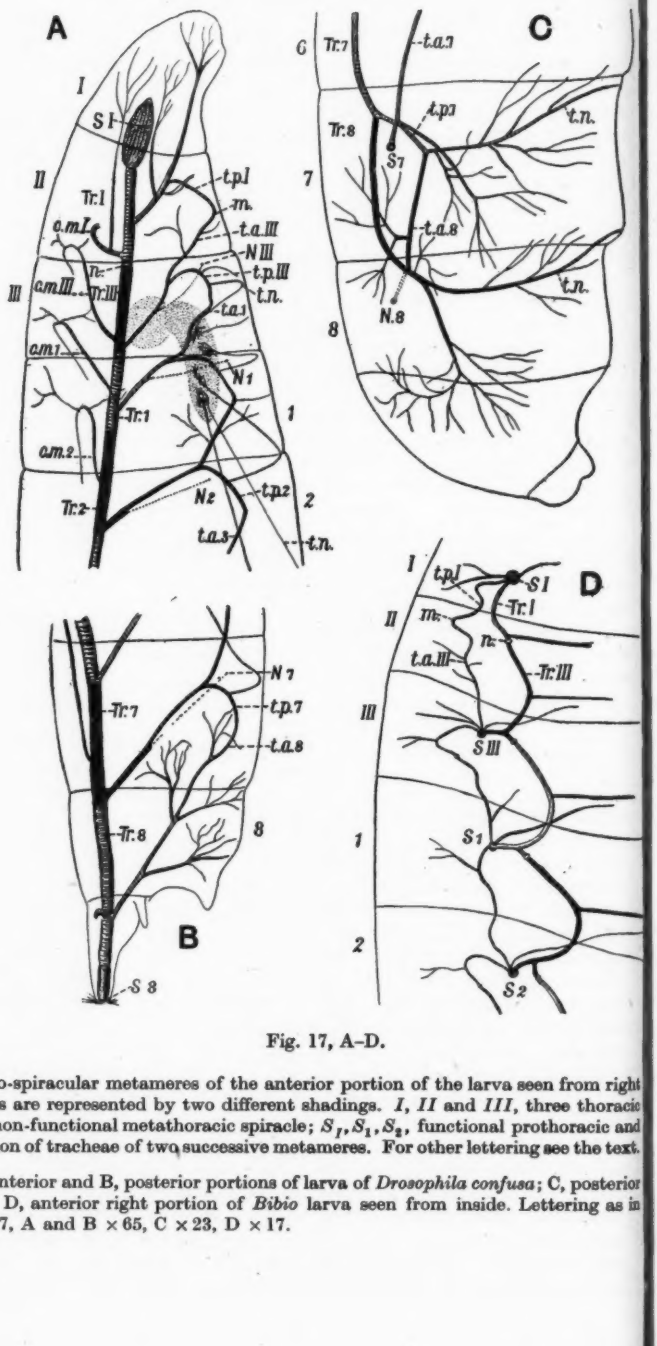


Fig. 17, A–D.

Fig. 16. *Mycetophila cingulum* Meig. Tracheo-spiracular metameres of the anterior portion of the larva seen from right side. The tracheae of successive metameres are represented by two different shadings. *I*, *II* and *III*, three thoracic and 1, 2, two abdominal segments; N_{III}, non-functional metathoracic spiracle; S_I, S_1, S_2, functional prothoracic and first two abdominal spiracles. *m.*, *n.*, junction of tracheae of two successive metameres. For other lettering see the text.

Fig. 17. Tracheo-spiracular metameres. A, anterior and B, posterior portions of larva of *Drosophila confusa*; C, posterior portion of larva of *Mycetophila cingulum*; D, anterior right portion of *Bibio* larva seen from inside. Lettering as in Fig. 16 and the text. Fig. 16 ×23, Fig. 17, A and B ×65, C ×23, D ×17.

(*b*) *Tracheo-spiracular metameres of holopneustic system*

The study of metamerism of the holopneustic system was carried out on the larvae of *Bibio* sp. As these larvae are not transparent, they were opened along their medio-ventral line, the integument unrolled and the viscera slightly displaced. Such a dissection (Fig. 17 D), even when examined under a low magnification, shows the metameric structure of the tracheal system which is easily recognized by small spherical swellings of the tracheae at the points of junction of metameres. Here also the two branches *Tr.I* and *t.p.I* of the first prothoracic spiracle (S_I) running posteriorly join, at points *n.* and *m.*, the

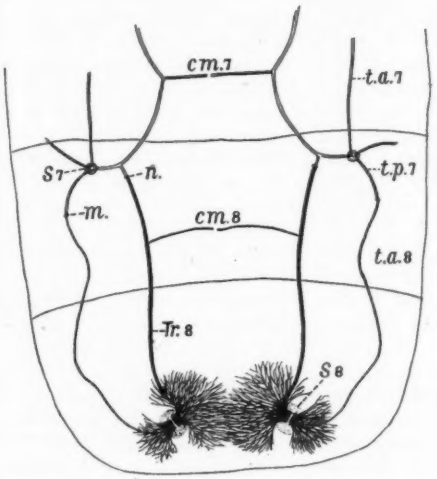


Fig. 18. Tracheo-spiracular metameres of posterior end of *Bibio* larva seen from inside. Letters as in Fig. 16 and the text. × 17.

branches *Tr.III* and *t.a.III* originating from metathoracic spiracle (S_{III}). The mesothoracic tracheae derive therefore from prothoracic (S_I) and metathoracic (S_{III}) tracheo-spiracular metameres.

The tracheae of the last abdominal segment (Fig. 18) derive from the last pair of abdominal spiracles (S_8). Their main branches (*Tr.* 8 and *t.a.* 8) supply also the major portion of the 7th abdominal segment.

(*c*) *Tracheo-spiracular metameres of peripneustic system*

The study of this metameric structure, carried out on different stages of the larvae of *Scatopse notata*, confirmed the results previously obtained, namely, that the larva has ten pairs of tracheo-spiracular metameres and that tracheal branches found in the mesothoracic segment derive from the prothoracic (S_I) and metathoracic (N_{III}) metameres.

In the course of this study it was found interesting to compare the peripneustic larvae of Diptera with those of other orders of insects. Among the latter the legless and transparent larvae of fleas (Aphaniptera) were frequently examined for the study of their internal organs. The opportunity was taken to examine in these larvae the metameric structure of their tracheal system. The larva has nine pairs of

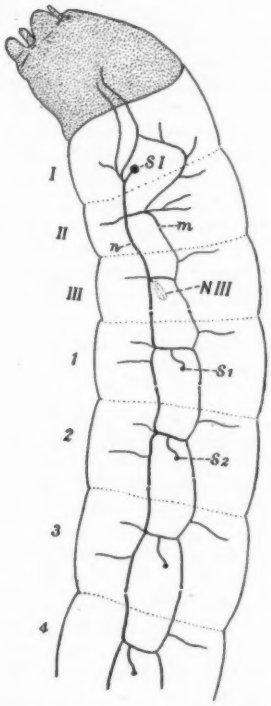


Fig. 19. Tracheo-spiracular metameres of a flea larva seen from the left side. The points of junction (*m.*, *n.*) between tracheae of successive metameres are marked schematically by breaks in tracheae. For explanation of other letters see Fig. 16.

functional and one pair of non-functional spiracles (S_I, N_{III}, S_{1-8}). The tracheal system was found to be composed of ten metameres, and, as it is shown in Fig. 19, the tracheae of the mesothoracic segment derive from prothoracic (S_I) and metathoracic (N_{III}) spiracles.

(*d*) *Tracheo-spiracular metameres of amphipneustic and metapneustic systems*

The metameric structure of these systems can be very easily seen in larvae of Anisopodidae (*Rhyphus*

and *Mycetobia*), Tipulidae, Dolichopodidae and others. It was interesting, however, to determine the metameres of the tracheal system in highly specialized larvae of cyclorrhaphous Diptera. The study of these larvae in spite of their transparency offers several difficulties. The fat-body is not distributed with regularity characteristic of orthorrhaphous larvae; and the alimentary tube being very long forms several loops occupying a large portion of the body. The nervous system being condensed into a single mass lying in the metathoracic and the first abdominal segments gives off long strands of nerves into every segment of the body and receives from them long branches of tracheae. Finally, the imaginal disks of the head and appendages are suspended on very long and thin peduncles. The body of such a larva when examined by transparency has therefore a very complicated appearance, each segment being traversed by long strands of nerves, tracheae and ligaments which makes very difficult the study of the tracheo-spiracular metameres and especially their connexions with the non-functional spiracles.

Nevertheless, it was found possible to carry out such a study on a living, young, third stage larva of *Drosophila confusa* Staeg. compressed between a slide and a cover-slip. The third stage larva is amphipneustic. The prothoracic spiracle (Fig. 17 A, S_1), which is well developed and composed of several finger-like processes, lies within a furrow and can be seen by transparency. Its position on the prothoracic segment can only be ascertained when the spiracle is evaginated. The postabdominal spiracle (Fig. 17 B, S_8) is supported by a short siphon. Careful examination of the junction between the tracheae shows that the tracheal system is composed of ten metameres, two of which originate from functional spiracles (S_1 and S_8) and eight from the non-functional spiracles (N_{III} and N_{1-7}). The non-functional spiracular branches are very long, thin filaments (represented in Fig. 17 A, B as dotted lines). Finally, as shown in Fig. 17 A, the tracheae of the mesothoracic segment derive from branches originating from the prothoracic (S_1) and metathoracic (N_{III}) spiracles.

An identical metameric structure of the tracheo-spiracular system was observed in the larvae of *Aulacogaster rufitarsis* and *Acidia heraclei*.

(e) *Tracheo-spiracular metameres of apneustic system*

The apneustic respiratory system was examined in ceratopogonine larvae of *Dasyhelea obscura* Winn. This larva also shows ten metameres originating in ten non-functional spiracles (N_1, N_{III}, N_{1-8}), and the tracheae in mesothoracic segment originate from the prothoracic and metathoracic non-functional spiracles (N_1 and N_{III}).

XI. GENERAL CONSIDERATIONS ARISING FROM THE STUDY OF TRACHEO-SPIRACULAR METAMERES

The respiratory systems of larvae of Diptera, Aphaniptera and probably the great majority of holometabolic insects, are composed of ten tracheo-spiracular metameres. The metameres originate from functional or non-functional spiracles which belong to the prothoracic, metathoracic and eight abdominal segments.

The mesothoracic segment is not only devoid of its pair of spiracles (functional or non-functional) but also of all tracheae which should belong to it; in other words, it is completely devoid of its tracheo-spiracular metamere, and the tracheae which are found within this segment derive from the prothoracic and metathoracic spiracles. The absence of a tracheal metamere in a well-developed segment intercalated between two other segments provided with such metameres, can be explained in two different ways: (1) either that the tracheal metamere proper to the mesothoracic segment did exist and gradually disappeared, or (2) that it never existed and its disappearance is therefore an apparent one.

(1) The first explanation can hardly be accepted since it is inconceivable that a tracheal metamere could disappear so completely in all larvae and all stages of their development without leaving any trace indicating its previous existence. Moreover, the respiratory requirements of the mesothoracic segment are at least as great as those of the metathoracic segment, since this segment has a normal structure in every other respect and in addition to larval organs contains the imaginal disks of the usual appendages (legs, wings and, in cyclorrhaphous larvae, also the head).

(2) The second explanation suggesting that an additional tracheal metamere proper to the mesothoracic segment never existed, is much more plausible. It is well known that the maximum number of segments (not counting the head) in dipterous larvae is eleven. Eleven is also the number of nerve ganglia in the ventral chain when the latter is not condensed. On the other hand, the maximum number of spiracles (functional and non-functional) and of the tracheo-spiracular metameres is ten. The only way to correlate the two constant numbers, namely, the eleven segments and ten pairs of spiracles, is to assume that the primary position of spiracles is intersegmental, as eleven segments provide only ten intersegmental spaces. The primitive form of an holopneustic respiratory system should therefore have ten pairs of intersegmental spiracles. The origin of real polypneustic systems as they are known to exist in different larvae can be explained by the migration of spiracles from the intersegmental membranes on to the segments. As there are only

ten pairs of spiracles, owing to such a migration one segment will be left without spiracles. Should the spiracles migrate in two opposite directions, the first pair moving anteriorly and the other nine pairs posteriorly, the spiracles would naturally appear upon the prothoracic, metathoracic and eight abdominal segments, and the mesothoracic segment would thus be deprived of spiracles. It would retain, however, the same tracheae, originating from the 1st and 2nd pairs of spiracles.

phila cingulum (Fig. 20 B, C) and *Mycetophila punctata* (Fig. 12 D, E, p. 14). We have seen, on the other hand, that the position of the spiracular openings relative to the scar indicates the direction of the proliferation of the new spiracle in relation to the old one. While the prothoracic spiracles, during the successive larval stages develop in front of old spiracles, the new abdominal spiracles are formed behind the old ones.

Fig. 20 E, which represents schematically a poly-

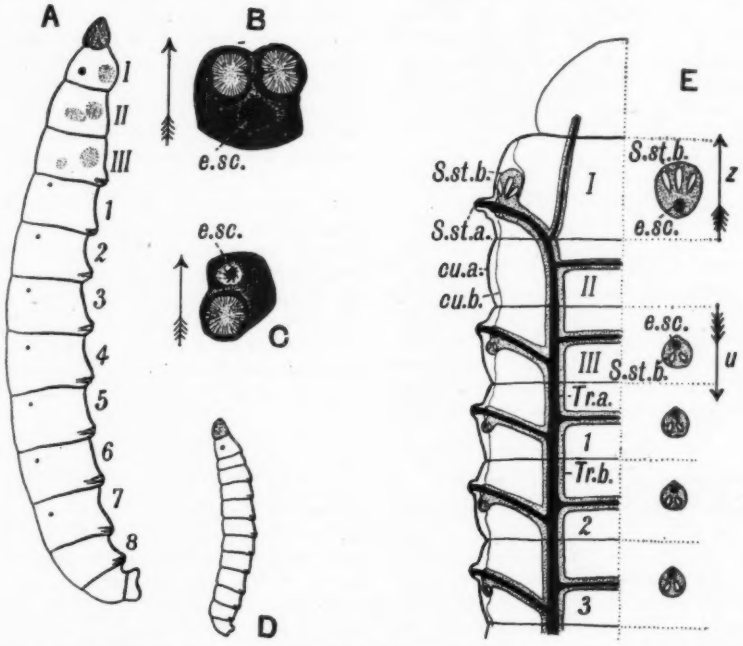


Fig. 20. *Mycetophila cingulum* (A–D). A, lateral view of a full-grown larva showing hemipneustic respiratory system; B, end view of prothoracic spiracle showing two spiracular papillae in front of external scar (*e.sc.*); C, end view of abdominal spiracle showing a single spiracular papilla behind the external scar; D, second stage larva of *M. cingulum* showing propneustic respiratory system; E, schematic figure representing the tracheo-spiracular system during the process of moulting separating stages *a* and *b*. For explanation of letters see the text (p. 25). A and D × 8, B and C × 400.

This explanation is strongly supported by the study of the structure and development of spiracles in the larvae of Mycetophilidae and Scatopsidae. One of the most striking differences between the prothoracic and the abdominal spiracles of these larvae* consists in the position of the spiracular openings relative to the scar. While the spiracular openings in prothoracic spiracles are situated anterior to the external scar (*e.sc.*), those of the abdominal spiracles lie posterior to the scar. This is clearly shown in the spiracles of the larva of *Mycetophila cingulum* (Fig. 20 B, C) and *Mycetophila punctata* (Fig. 12 D, E, p. 14). We have seen, on the other hand, that the position of the spiracular openings relative to the scar indicates the direction of the proliferation of the new spiracle in relation to the old one. While the prothoracic spiracles, during the successive larval stages develop in front of old spiracles, the new abdominal spiracles are formed behind the old ones.

* Except the postabdominal spiracles of scatopsid larvae.

pneustic respiratory system during the process of moulting separating two larval stages (*a* and *b*), clearly explains the phenomenon discussed above. The two superimposed larval cuticles (*cu.a.* and *cu.b.*) are connected with their tracheae (*Tr.a.* and *Tr.b.*). The new prothoracic spiracle of stage *b* (*S.st.b.*) is formed anterior to the old one (*S.st.a.*), while the abdominal spiracles of stage *b* are formed posterior to those of stage *a*. On the right of this schematic figure the spiracles of stage *b* are represented endwise after the moult and the formation of an external scar (*e.sc.*). The two arrows *z* and *u* indicate the direction of proliferation of spiracular

epithelium during the successive moults. It is therefore highly probable that anterior proliferation of the prothoracic spiracles and posterior proliferation of abdominal spiracles are intimately connected with the migration of corresponding spiracles in opposite directions and the absence of spiracles on the mesothoracic segment.

Finally, the theory of the intersegmental origin of spiracles explains Palmén's observation confirmed by Krancher (1881, p. 512) and other workers, namely, that the prothoracic and mesothoracic spiracles exclude each other. In fact, it is the same pair of spiracles lying originally between the first two thoracic segments and migrating either anteriorly or posteriorly to become a prothoracic or mesothoracic pair of spiracles.

XII. DEVELOPMENT OF RESPIRATORY SYSTEMS DURING SUCCESSIVE LARVAL STAGES

The larvae of Diptera pass through several stages of development separated by two, three or more moults, and accompanied not only by growth but also by marked morphological changes. The study of changes in the respiratory organs is of special interest and will be examined for three respiratory systems: peripneustic, hemipneustic and amphipneustic.

(*a*) *Development of peripneustic system*

The respiratory system of a peripneustic larva of *Scatopse notata* (Fig. 21 A) shows nine pairs of functional (S_I, S_{1-8}) and one pair of metathoracic non-functional (N_{III}) spiracles. The prothoracic spiracles (D, G) are well developed and are composed of 7–8 papillae lying in front of the scar while the first seven pairs of abdominal spiracles are less prominent and have 4–5 papillae lying behind the scar (H, I). The postabdominal spiracles (Fig. 21 B, C, S_8 and F) are supported by two long hard horns arising from the dorsal side of the last abdominal segment. Each spiracle is composed of numerous clefts radiating around the central scar plug.

The complete development of this fly was readily observed, as mating takes place easily in captivity and females lay their eggs on different decomposed vegetable substances especially the mangold wurzel. Each egg is surrounded by a gelatinous layer which swells when submerged in water. The larva when ready to hatch can be seen by transparency with its segments telescoped into each other.

Stage I. The larva hatches with the tracheae filled with air. The respiratory system is metapneustic with only one postabdominal pair of functional spiracles, supported by a pair of short horns (Fig. 22 A, S_8). All other spiracles are non-functional and appear as small transparent papillae connected with lumenless spiracular branches, in the prothoracic, metathoracic and seven abdominal segments.

Stage II. At this stage the larva is amphipneustic (Fig. 22 B) showing two pairs of functional spiracles, postabdominal (S_8) and prothoracic composed of three papillae (S_I). The remaining eight pairs which are non-functional are connected with the tracheal system by means of a lumenless scar filament (Fig. 22 C, *Sc.f.*).

Stage III. The larva now becomes peripneustic with nine pairs of functional spiracles (one prothoracic and eight abdominal) and one pair (metathoracic) non-functional. The prothoracic spiracles are larger than in the previous stage and composed of 4–5 papillae. The formation of a functional abdominal spiracle from a non-functional of the previous stage is clearly shown in Fig. 22 D.

Stage IV. The larva is again peripneustic. The prothoracic spiracles are composed of 7–8 papillae while the first seven abdominal spiracles comprise four or five papillae. The process of moulting of a spiracle separating stages III and IV is clearly shown in Fig. 22 E. On a few occasions one of the abdominal spiracles of stage III was found non-functional. However, during the next moult it gave rise to a normal and well-developed spiracle of stage IV (Fig. 22 F).

(*b*) *Development of hemipneustic system*

The study of the development of this system was carried out on the larvae of *Mycetophila cingulum*. The tracheal system and spiracles of a full-grown larva of this species were previously described and figured (cf. pp. 21, 25 and Figs. 16, 17 C, 20 A–D).

Stage I. The larva after emergence from the egg already has its thin tracheae filled with air. Its respiratory system is metapneustic (Fig. 23 A) and has only one pair of functional spiracles on the eighth abdominal segment (Fig. 23 A, B, S_8). This pair of spiracles is very small and only slightly protruding from the surface of the body. It is, however, clearly visible because of the air which fills its felt chamber and its spiracular branch. The other spiracles, namely, the prothoracic, metathoracic and the seven abdominal, are non-functional.

This larval stage, being of very short duration, can be easily overlooked. The hypoderm of the integument and the tracheal epithelium, especially around the non-functional prothoracic spiracles (Fig. 23 C), are found in a state of proliferation, and only a few hours after hatching from the egg the larva moults and passes into the next stage.

Stage II. The larva appears to become propneustic. The prothoracic spiracles are very small

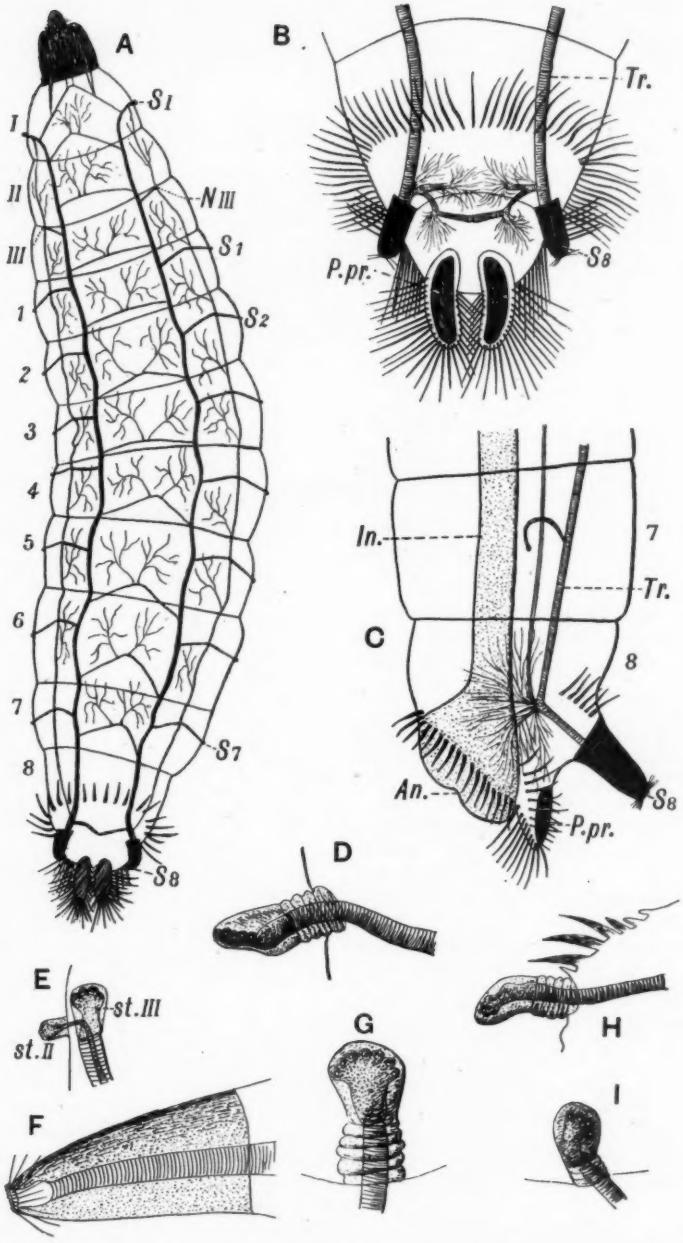


Fig. 21. *Scatopse notata*. Respiratory system of the larva. A, dorsal view of a full-grown larva showing the tracheo-spiracular system; B, dorsal view of postabdominal segment; C, lateral view of the last abdominal segments; D, side view of prothoracic spiracle; E, prothoracic spiracle undergoing a moult separating second and third larval stages; F, postabdominal spiracular siphon; G, prothoracic spiracle, front view; H, I, abdominal spiracle, side and front views. *I*, *II* and *III*, three thoracic and 1–8, eight abdominal segments; *S*, functional and *N*, non-functional spiracles; *P.pr.* postabdominal processes. A × 23, B–C × 55, D–I × 260.

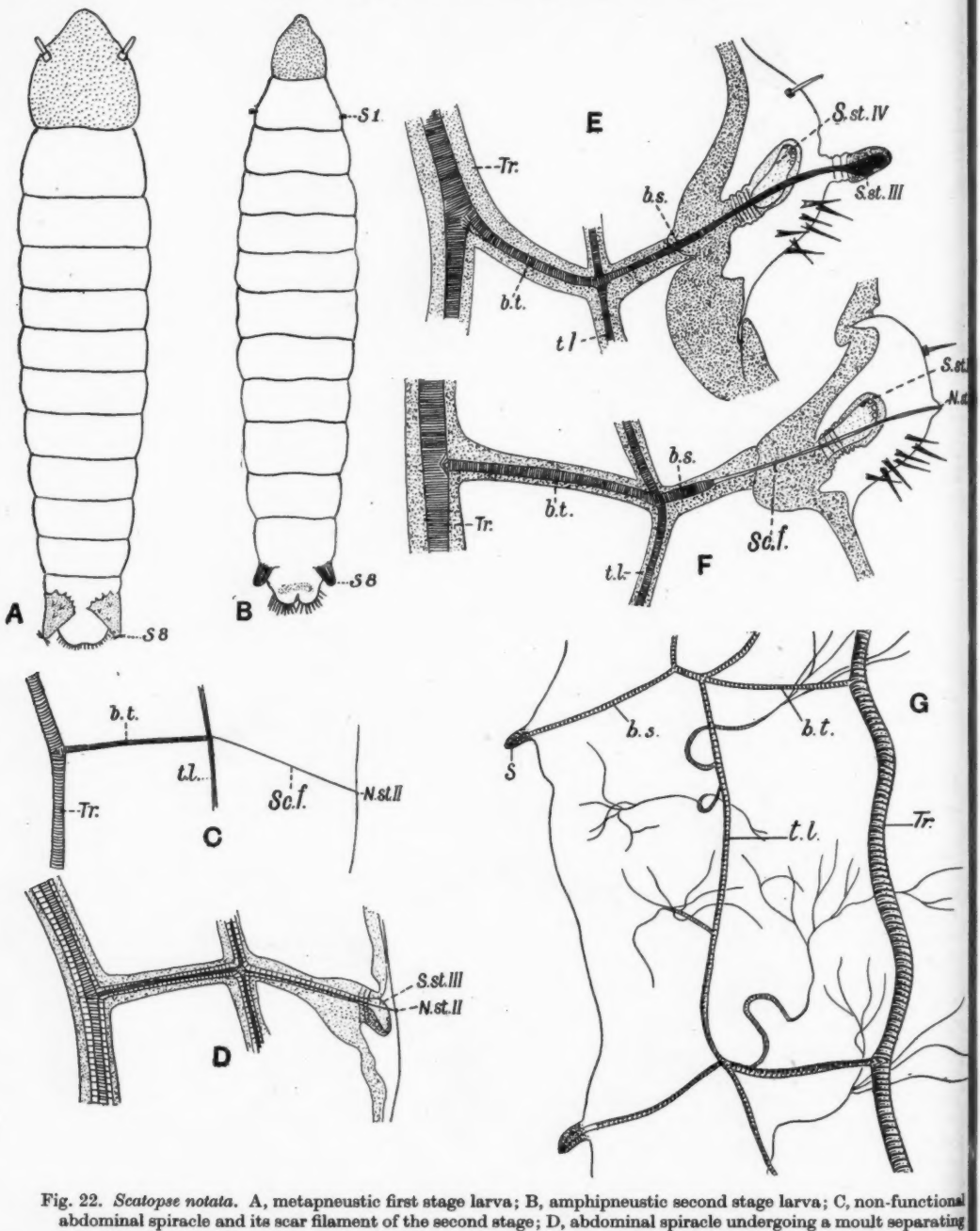


Fig. 22. *Scatopse notata*. A, metapneustic first stage larva; B, amphipneustic second stage larva; C, non-functional abdominal spiracle and its scar filament of the second stage; D, abdominal spiracle undergoing a moult separating second and third stages; E and F, abdominal spiracles undergoing a moult separating stages III and IV. For F, see the text (p. 26); G, portion of tracheo-spiracular system of a full-grown larva. *b.t.* transverse branch; *b.s.* spiracular branch; *N*, non-functional; *S*, functional spiracles; *Sc.f.* scar filament; *t.l.* longitudinal latero-ventral trunk; *Tr.* latero-dorsal trunks. A ×175, B ×55, C–F ×260, G ×175.

but connected with the longitudinal trunks by means of a short tracheal branch devoid of the felt chamber (Fig. 24 A, *a*). It is, however, important to note that this spiracular branch was usually found to contain liquid instead of air, thus making it impossible to decide whether the spiracle is already functional. The postabdominal spiracle which was previously functional is now completely closed and connected with the tracheal trunk by means of a typical scar filament. All the other spiracles remain non-functional. This larval stage is also of very short duration and its peritracheal epithelium rapidly proliferates, as can be seen on examination of its spiracles and their branches in the prothoracic (Fig. 24 A), metathoracic (B), one of the seven abdominal (C) and the last abdominal (D) segments.

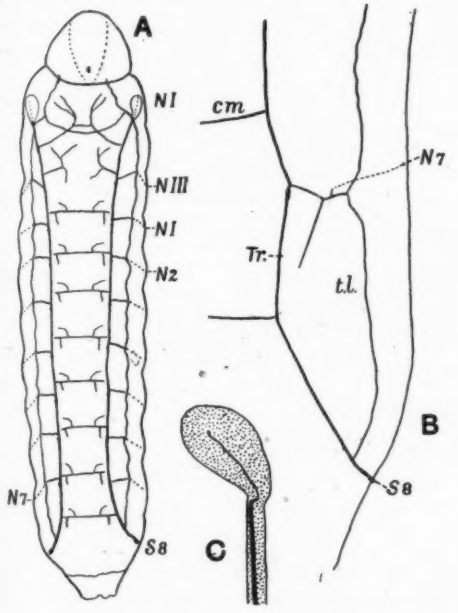


Fig. 23. *Mycetophila cingulum*. A, metapneustic first stage larva; B, tracheo-spiracular system of its last abdominal segments; C, prothoracic non-functional spiracle undergoing a moult. A × 87, B and C × 400.

Stage III. The larva now becomes distinctly propneustic with the prothoracic spiracles well developed (Figs. 20 D, 24 A, *S.st.III* and E). The remaining nine pairs of spiracles are non-functional. This stage is of longer duration and can easily be seen among the young larvae of different mycetophilids. The tracheal epithelium of the larvae soon begins to proliferate (Fig. 24 E, F, G and H) to form the functional and non-functional spiracles of the next stage.

Stage IV. The larva now becomes hemipneustic (Fig. 20 A). The prothoracic spiracles (Fig. 20 B) become larger and show two papillae lying in front of an external scar (*e.sc.*). The first seven pairs of abdominal spiracles are functional and composed of one papilla (C) lying behind the scar. One of these spiracles during the process of moulting of the third stage is shown in Fig. 24 H. The metathoracic and the 8th abdominal spiracles remain non-functional.

Certain of these stages were ultimately confirmed on other mycetophilid larvae, the material of which was not, however, sufficient for the study of the complete life history. Thus among the larvae of *Sciara* sp., living upon damaged potatoes, were found the metapneustic, propneustic and hemipneustic stages, and in the larvae of *Plastosciara keilini* Edwards which live in rotten wood the stages observed were apneustic, propneustic and hemipneustic; the metapneustic stage which is of a very short duration was probably overlooked. The propneustic and hemipneustic stages were observed in many other mycetophilid larvae.

(c) *Development of amphipneustic system*

(1) *Diptera cyclorrhapha*

It is well known that full-grown larvae of cyclorrhaphous Diptera are generally amphipneustic and pass in their development through three stages separated by two moults:

Stage I is usually metapneustic with one postabdominal pair of spiracles functional and nine pairs of non-functional spiracles (prothoracic, metathoracic and seven abdominal).

Stage II is amphipneustic: the prothoracic and postabdominal pairs are functional while the metathoracic and the first seven abdominal pairs are non-functional.

Stage III remains amphipneustic but with more developed tracheae and spiracles. The process of moulting of spiracles has been already described (p. 6) and illustrated (Figs. 4 E–H, 11 A and 12 C).

The above-mentioned three stages of the respiratory system are met with in the great majority of cyclorrhaphous dipterous larvae; there are, however, a few exceptions which can be examined under the following four groups:

(i) Larvae metapneustic in stages I and II, but becoming amphipneustic in stage III. Examples: *Thrixion halidayanum* Rond. described by Pantel (1898) and *Panzeria rudis* Fall. described by Nielsen (1909).

(ii) Larvae metapneustic in all three stages. Examples: *Ocyptera brassicaria* F. according to Nielsen (1909) and the larvae of several leaf-mining ephydrines: *Hydrellia modesta* L. and *Notiphila dorsata* Stenk. It is interesting to note that these two larvae show the remains of the felt chambers of the

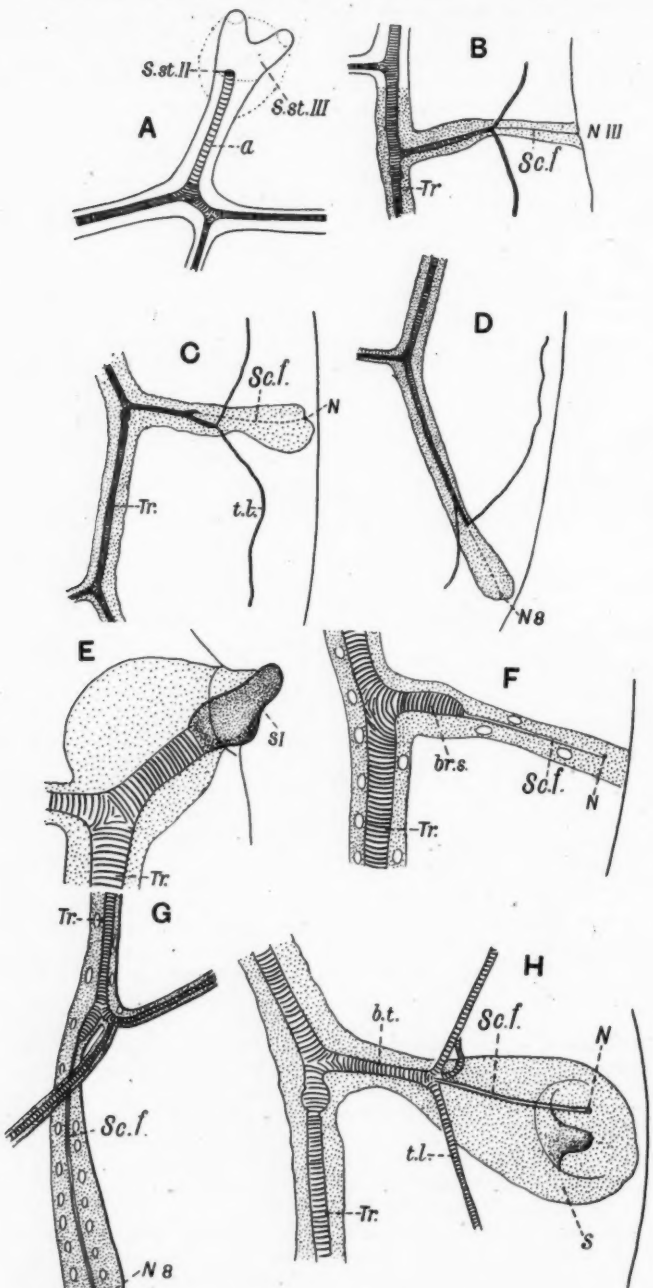


Fig. 24. *Mycetophila cingulum*. A, prothoracic; B, metathoracic; C, abdominal; D, last abdominal spiracles undergoing a moult separating larval stages II and III; E, prothoracic spiracle of stage III; F, metathoracic; G, eighth abdominal and *H*, first abdominal spiracles passing from stage III to stage IV; letters as in previous figures. × 400.

prothoracic spiracles (Fig. 12 G, *f.ch.*, p. 14) thus indicating that these spiracles in the second and third stage larvae have only recently become non-functional.

(iii) Larvae amphipneustic in all three stages. Examples: a number of carnivorous larvae of anthomyids such as *Mydaea urbana* Meig. and *Myospila meditabunda* F. (Portchinsky, 1910), *Melanochelia riparia* Fall. (Keilin, 1917), *Mydaea pagana* Fall. and *Phaonia variegata* Meig. (Thomson, 1937). It should be noted that at least in a few of these cases there is no evidence of the existence of a moult separating the larva emerged from an egg and the full-grown larva ready to pupate (Keilin, 1917; Tate, 1935).

(iv) Larvae apneustic in stage I and amphipneustic in stage II. Example: a pipunculid larva of *Ateleneura spuria* Meig. parasitic in *Typhlocyba* (Homoptera) as described by Keilin & Thompson (1915).

(2) *Diptera Orthorrhapha*

The amphipneustic larvae of *Trichocera hiemalis* Meig., *Anisopus fenestralis* Scop., *Mycetobia pallipes* Meig., *Psychoda phalaenoides* L. and many others investigated pass through a metapneustic stage. The metapneustic larvae of Tipulidae and Culicidae and the apneustic larvae of Chironomidae and Simulidae are metapneustic and apneustic respectively in all their larval stages.

So far as the propneustic larvae of certain mycetophilids are concerned, we possess unfortunately no information as to the structure of their early larval stages.

XIII. CONCLUSIONS DEDUCED FROM THE STUDY OF THE DEVELOPMENT OF RESPIRATORY SYSTEMS

The study of the development of different respiratory systems, the main results of which are summarized in Fig. 25, leads to the following generalizations:

(1) The tracheo-spiracular apparatus of the larva is already complete at the time of its hatching from the egg, i.e. all the tracheo-spiracular metameres are present, arising from either functional or non-functional spiracles.

(2) In all stages, the total number of spiracles and of the tracheo-spiracular metameres is ten pairs, and this applies to all the respiratory systems.

(3) The spiracles which become functional after a moult, are always formed from pre-existing spiracles and their branches, which may or may not have been functional; no spiracle ever appears independently of a corresponding spiracle of the previous stage.

(4) The metathoracic spiracles of dipterous larvae, except those of Bibionidae, remain non-functional in all stages of the larva; however, they become functional in adult flies.

(5) The polypneustic larvae pass during their development through oligopneustic stages.

(6) The mesothoracic spiracles together with their tracheal metameres are absent in all larval stages.

(7) There are no transition stages between the oligopneustic and polypneustic systems during the development of the larva. The seven pairs of abdominal spiracles are either all functional or all closed.

(8) The metapneustic system appears as the first stage of development in all respiratory systems except the apneustic one.

(9) The fact that the metapneustic system occurs also in the development of the hemipneustic system is of special interest. The appearance of a pair of postabdominal functional spiracles for a short period in mycetophilid larvae shows first of all that the hemipneustic respiratory system derives from the peripneustic system. It shows also that the closed non-functional spiracle ought not to be considered as a primitive rudiment of an organ in development but as a secondary rudimentary form of organ derived phylogenetically from a normal functional spiracle.

(10) The development of the hemipneustic system differs from that of other polypneustic systems by passing through a propneustic stage.

(11) In the course of development of different respiratory systems, the functional spiracles appear in the order of their morphological and physiological importance. If the relative importance of spiracles in a peripneustic system can be expressed as $S_8 > S_1 > S_{1-7}$, the successive stages of development are: metapneustic, amphipneustic and peripneustic. The only exception to this rule is the appearance, for only a very short period, of a postabdominal spiracle in the development of hemipneustic larvae of mycetophilids.

The generalizations (5) and (11) appear to contradict the biogenetic law of recapitulation, according to which the meta- and amphipneustic systems which appear as the first two stages in the development of the peripneustic system should be considered as ancestral and more primitive systems from which the peripneustic system is derived. However, no entomologist would agree that this is the case. On the contrary, it is generally accepted that meta- and amphipneustic forms which are adapted to a partially submerged condition of life are more specialized and are derived from the primitive terrestrial polypneustic form.

On the other hand, the appearance of a pair of postabdominal spiracles during a short period of development in the larva of mycetophilids which

are devoid of these spiracles is an excellent illustration of the law of recapitulation, considering that the hemipneustic system must have originated from a more primitive peripneustic system.

Segments	I	II	III	1	2	3	4	5	6	7	8	
1 Holopn. pr.	○	○	○	○	○	○	○	○	○	○		
2 Holopn.	○		○	○	○	○	○	○	○	○	○	Polypneustic.
3 Peripn.	○		•	○	○	○	○	○	○	○	○	
4 Hemipn.	○		•	○	○	○	○	○	○	○	•	
5 Amphipn.	○		•	•	•	•	•	•	•	•	○	Oligopn.
6 Metapn.	•		•	•	•	•	•	•	•	•	○	
7 Propn.	○		•	•	•	•	•	•	•	•	•	
8 Apn.	•		•	•	•	•	•	•	•	•	•	Apn

A

○ funct. S • non funct. N

Segments	I	II	III	1	2	3	4	5	6	7	8
Stage I	•		•	•	•	•	•	•	•	•	○
" II	○		•	•	•	•	•	•	•	•	○
" III	○		•	○	○	○	○	○	○	○	○
" IV	○		•	○	○	○	○	○	○	○	○

B

Segments	I	II	III	1	2	3	4	5	6	7	8
Stage I	•		•	•	•	•	•	•	•	•	○
" II	◎		•	•	•	•	•	•	•	•	•
" III	○		•	•	•	•	•	•	•	•	•
" IV	○		•	○	○	○	○	○	○	○	•

C

Segments	I	II	III	1	2	3	4	5	6	7	8
Stage I	•		•	•	•	•	•	•	•	•	○
" II	○		•	•	•	•	•	•	•	•	○
" III	○		•	•	•	•	•	•	•	•	○

D

Fig. 25. Schematic figures representing the distribution of functional (*S*) and non-functional (*N*) spiracles; A, in different respiratory systems; and in successive stages of B, peripneustic; C, hemipneustic and D, amphipneustic larvae. I, II and III—three thoracic and 1–8—eight abdominal segments.

PART 2. RESPIRATORY ADAPTATIONS OF LARVAE AND PUPAE OF DIPTERA

The great diversity of conditions under which dipterous larvae live and develop was already referred to in the introduction to this paper. It suggests that the respiratory requirements of these larvae may greatly affect their behaviour, their life history and even their structure. The study of these relationships, which can be described as the respiratory adaptations of the larvae, will be the main subject of the next few sections of this paper. At this point I should like to remind the reader once again that the biochemical and physiological aspects of insect respiration are excluded from these as from the previous sections. They will be dealt with separately in a series of papers specially devoted to these subjects.

XIV. RESPIRATORY ADAPTATIONS OF AQUATIC LARVAE

Of all the larvae of Diptera, those which are adapted to aquatic life are by far the best known. The literature dealing with these larvae is a very extensive one and goes far back to the work of Swammerdam, Réaumur and De Geer. From the beginning of this century the interest in aquatic dipterous larvae was greatly stimulated by three independent factors. The first and certainly the most important factor was the discovery that mosquitoes act as vectors in the transmission of human diseases such as malaria, yellow fever and filariasis.

The second factor is found in the investigations which have established the importance of dipterous larvae as food for fresh-water fish. The third factor was the appearance of Miall's (1895) delightful book, *The Natural History of Aquatic Insects*, which, together with several other monographic studies published by him in collaboration with other workers, attracted many fresh investigators to this field of biology.

Much important information concerning the adaptations and structures of aquatic dipterous larvae can be found collected in papers by Malloch (1915, 1917), Wesenberg-Lund (1915), Alexander (1920) and the important memoirs by Johannsen (1934, 1935, 1937 and 1937*a*).

According to their respiratory systems the aquatic dipterous larvae can be separated into two main groups: (*a*) *apneustic* larvae: Chironomidae, Simulidae, Blepharoceridae, *Chaoborus* among Culicidae and *Antocha* among Tipulidae; (*b*) *oligopneustic* (amphi- and metapneustic) larvae: Ptychopteridae, Tipulidae, Psychodidae, Culicidae, Thaumaleidae, Stratiomyidae, Tabanidae, Syrphidae, Tetanoceridae, Ephydridae and representatives of a few other families of Diptera Cyclorrhapha.

The *apneustic* larvae of Chironomidae are usually found in lakes, ponds and sluggish streams. Only a few species of *Tanytarsus* are found in rapidly flowing water. The larvae are found either free swimming in water or enclosed in different kinds of tubes which they build as in the case of different species of *Tanytarsus* (Fig. 26 A–C) (see Lauterborn, 1905; Thienemann, 1910; and Bause, 1913). In these tubes

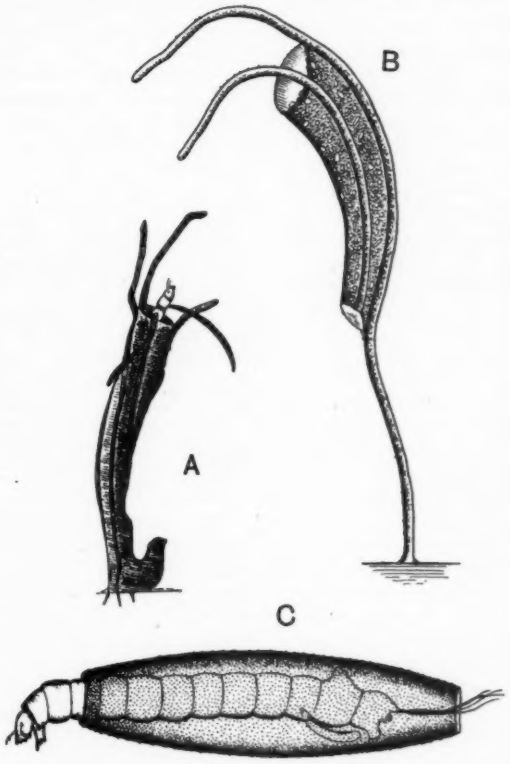


Fig. 26. Cases or tubes of certain larvae of Chironomidae from Bause (1913). A, *Tanytarsus pentapoda* (after Thienemann, 1910); B, *Tanytarsus exiguus* (after Lauterborn, 1905); C, *Tanytarsus flexilis* (after Lauterborn, 1905). A and B × 6·6, C × 10.

the body of the larvae by oscillating movement sets up a current of water which seems to assure its respiratory requirements. The larva is able to leave its case and, after looping through the water in a characteristic way, to return to the case (Miall & Hammond, 1900). The tracheal system of apneustic larvae is usually not well developed. In

certain forms it appears only in later larval stages and is incomplete, being filled with air only in a few anterior segments. The larvae in several species are provided with thin tube-like extensions of the body wall filled with blood and known as blood gills. In *Chironomus dorsalis* L. and *plumosus* Mg. there are two kinds of these 'gills'. Two pairs of 'ventral gills' originating on the ventral side of the 8th abdominal segment and two pairs of 'anal gills'. So far there is no indication that under normal conditions these 'blood gills' have any special function in respiration (Fox, 1921). They may, however, play a more active part in respiration during the period of recovery from lack of oxygen (Harnisch, 1930).

The blood of some chironomid larvae contains haemoglobin which plays an important part as an oxygen carrier when the tension of oxygen in the surrounding medium falls below a definite level,* varying according to the species examined from 1 to 7 %. It is interesting to note that according to Pause (1919) young larvae of *Ch. gregarius* are colourless and devoid of tracheae until they reach the size of about 3·5 mm.; haemoglobin and tracheae appear gradually, and in distinctly or deeply red larvae 6·6–15 mm. long, the tracheal system reaches the 3rd segment of the body. While the larvae are young and devoid of haemoglobin they are strongly phototropic, being always attracted by well-illuminated water rich in water plants and oxygen. With the development of haemoglobin their phototropism gradually becomes negative and they appear as more constant inhabitants of mud. Interesting and yet unexplained are some of the observations carried out by Cole (1921) on *Ch. tetans* found at the bottom of the lake Mendota. It was previously shown by Birge (1907) that during summer months when the upper layer of water becomes warm and light the wind is unable to mix the water from top to bottom of the lake, and the water undergoes a gradual stratification, the bottom layer, the hypolymnion, becoming stagnant and devoid of oxygen for at least 3–4 months. During this period most of the animals migrate from the hypo- to the epilymnion. However, a few organisms including the larvae of *Ch. tetans* remain at the bottom of the lake where they seem to have a very active life. It is difficult to explain how haemoglobin or blood gills can be used by the larvae under these almost completely anaerobic conditions of life.

Blepharoceridae. The apneustic larvae of Blepharoceridae are found attached to rocks or stones in highly aerated rushing water of creeks, brooks, streams and waterfalls such as are found in hilly or mountainous regions. The larvae are flat and the body is highly modified in shape, being divided into seven sections, the first being composed of the head, thorax and the first abdominal segment, then follow five abdominal segments and a section composed of at least two abdominal segments. The ventral side of the body shows six strong median suckers which enable the larva to attach itself to rocks or stones. The abdominal segments are provided with about five pairs of tufts of finger-like processes or filament, three or more per tuft, described as 'gills'. We have no evidence yet as to the respiratory function of these tufts.

Simulidae (black flies or buffalo gnats). The larvae of Simulidae are usually found in swiftly running streams or rivers. They have sedentary habits, being attached in an upright position, by means of a sucker-like disk of their posterior end, to smooth stones or plants submerged in water. The larvae are very sensitive to oxygen and will always move towards the spot richest in oxygen, for instance, highly aerated places especially near bubbles of air. Although apneustic, they have a very well-developed tracheal system (Puri, 1925, see his fig. 7, pl. VIII), the larval body wall being richly supplied with a highly ramified net of tracheoles. The larvae are provided with finger-like branched retractile anal processes supplied with fine single tracheoles. These 'blood and tracheal gills' probably have a respiratory function.

Oligopneustic larvae. Several large families of Diptera have meta- or amphipneustic larvae adapted to an aquatic or semi-aquatic mode of life. The respiratory activity of these larvae depend almost entirely on the contact between their postabdominal spiracles and the atmospheric air. As their food supply is usually found in deeper layers of water, the contact between the spiracles and the air can be only of an intermittent nature. The methods of establishing such contacts vary from one species to another and provide perhaps the most interesting examples of respiratory adaptations of larvae. The main peculiarities of these adaptations can better be examined under the following headings: (1) posterior terminal spiracular disks; (2) postabdominal respiratory siphons; (3) plant perforating spiracles; (4) perispiracular pneumatic sacs; (5) certain modifications of the tracheal system.

(1) *Posterior terminal spiracular disk.* In a large number of species of Tipulidae and Anisopodidae the posterior end of the larva is usually truncated forming a disk bearing the postabdominal spiracles and surrounded by two to eight fleshy lobes often strengthened on the inner surface with black sclerotized plates and fringed with hairs which may become very long. The disk, lobes and hairs surrounding them, being covered by the hydrofuge secretion of perispiracular glands, in contact with the water surface expand by the surface tension and so bring the spiracles in contact with air. When the

* The respiratory function of haemoglobin in chironomids, as well as other larvae, will be discussed in a special paper.

larva is completely submerged the disk is drawn inside and the fleshy lobes close over the spiracular disk, protecting it from contact with water. The variety in structure of spiracular disks is of taxonomic value and a large number of interesting examples were described and figured by Alexander (1920) for the larvae of Tipulidae.

(2) *Postabdominal respiratory siphons.* The postabdominal spiracles of the larva are often supported by a more or less elongated projection or siphon of the last abdominal segment. In certain families there is a complete series of transitions between the forms provided with sessile spiracles and those having a very long siphon. The best examples of the latter are found in the larvae of ptychopterids and eristalines. In both cases the siphon is retractile and adapted to varying depths of water. The respiratory system of *Ptychoptera* was well described by Grobben (1875) and by Miall (1895), while siphons of different ptychopterids are described and figured by Alexander (1920). In the larvae of *Eristalis* (Réaumure, 1738; Batelli, 1879; Miall, 1895; and Gäbler, 1930) the siphon is exceptionally long (Fig. 27 B) and the spiracles can reach the surface of the water even when the larva is feeding at a depth of 15 cm. The fully extended siphon is seven times longer than the body of the larva. It is composed of three portions which, by contraction of special muscles, can be telescoped, thus reducing its length to the depth of the water layer covering the larva. In other larvae of syrphids such as *Ceria conopsoides* L. (Fig. 27 A), *Xylota pigra* Fab., *Eumerus strigatus* Fln. and *Syritta pipiens* L., the siphon is not contractile and is progressively shorter than in *Eristalis* larvae.

In the larvae of *Melanochelia riparia* (Fig. 28 A) among the Anthomyidae and the larvae of Ephydridae (Fig. 28 B) each of the postabdominal spiracles is supported by an independent siphon or a branch of a common siphon bifurcated terminally.

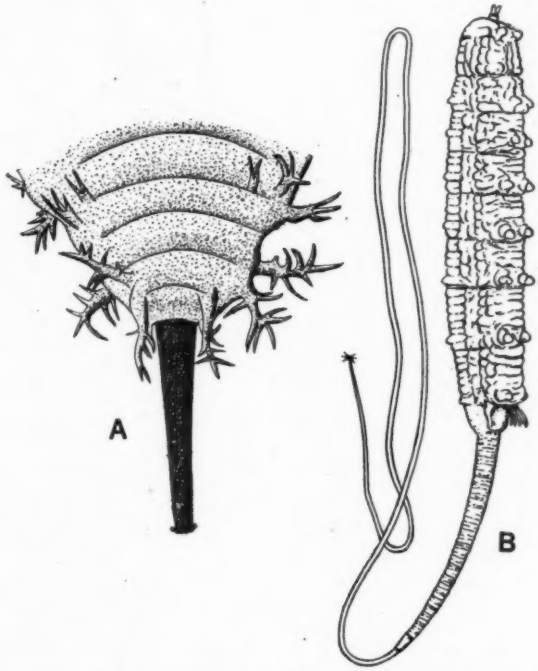
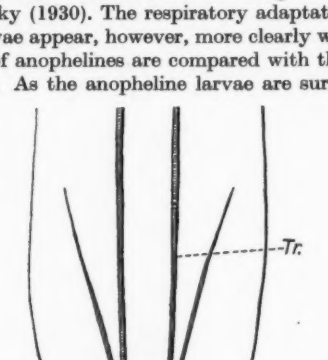


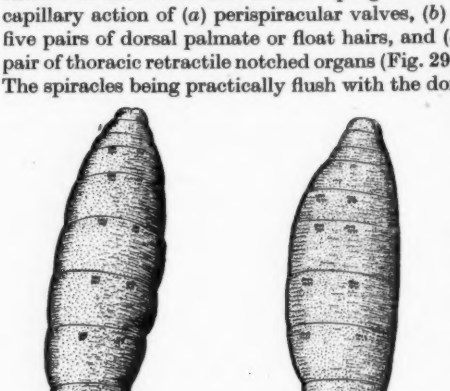
Fig. 27. Respiratory siphons in syrphid larvae. A, *Ceria conopsoides* larva, posterior portion of the body; B, *Eristalis tenax* larva (B after Berlese, 1909). A × 21.

The most interesting and perhaps the best known examples of respiratory adaptations are found among the larvae of Culicidae. These larvae are metapneustic, having their postabdominal spiracles open and surrounded by five more or less developed fleshy perispiracular valves. These valves, which are covered by a hydrofuge secretion from perispiracular glands (Keilin *et al.* 1935) can be either folded over the spiracles, protecting them in a diving larva from water intrusion, or can be expanded in the plane of the water surface, thus bringing the spiracles in contact

with air. The structural variations of this organ in different species were described and figured by Montschadsky (1930). The respiratory adaptations of these larvae appear, however, more clearly when the larvae of anophelines are compared with those of culicines. As the anopheline larvae are surface feeders, the body lies horizontally parallel with the surface of the water and is held up against it by capillary action of (*a*) perispiracular valves, (*b*) the five pairs of dorsal palmate or float hairs, and (*c*) a pair of thoracic retractile notched organs (Fig. 29 A). The spiracles being practically flush with the dorsal



28 A



28 B

Fig. 28. A, *Melanochelia riparia*, posterior portion of the body; B, *Ephydra riparia* larvae attached to roots of plants. *Co.p.* posterior commissure; *Sp.* spiracles; *Tr.* tracheal trunks. A ×24, B ×8.

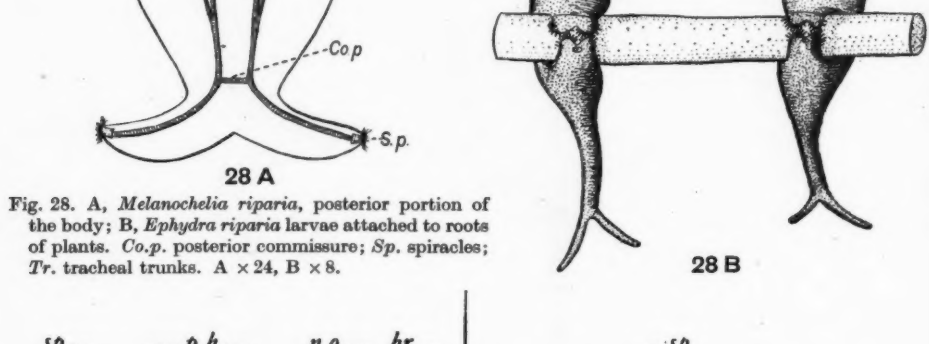


Fig. 29. Larvae and pupae of mosquitoes. Schematic figures after Marshall (1942). A, *Anopheles*. B, *Culex*; *a.g.* anal gills; *an.* antenna; *br.* mouth brushes; *e.* eye; *h.h.* hooked hairs; *n.o.* notched organ; *p.h.* palmate hairs; 1 *sg.* 1st abdominal segment; 8 *sg.* 8th abdominal segment; *si.* siphon; *sp.* spiracles; *th.* thorax; *tr.* breathing trumpets; *w.s.* water surface.

surface of the 8th abdominal segment are thus in contact with the air. On the other hand, as in this position the larva lies with the dorsal side of the head uppermost, in order to feed upon organisms or debris floating upon or just under the water surface, the larva rotates its head through an angle of 180°. The culicine larvae are capable of feeding at different depths including the bottom of the water reservoir. Their postabdominal spiracles are supported by a more or less long siphon which projects dorsally from the last abdominal segment and the larva hangs head downwards, being supported by the capillary action of the perispiracular valves (Fig. 29 B).

(3) *Plant-perforating spiracles.* Larvae capable of obtaining air from aquatic plants are now known among several families of Diptera. The best example of this habit is that of a mosquito larva, *Taeniorhynchus richiardii*, the detailed study of which we owe to Wesenberg-Lund (1918) and Edwards (1919). The larva of *T. richiardii* is adapted to a completely submerged mode of life, obtaining oxygen from roots of aquatic plants by means of a postabdominal spiracular siphon modified into a perforating organ (Fig. 30 A–C). The larva was found attached to the submerged roots of the following plants: *Acorus*, *Glycera*, *Hydrocharis*, *Ranunculus* and *Typha*. The mechanism of the perforation of plant roots by the spiracles of the larva was studied by Edwards (1919, see pp. 85–6) and described by him as follows (Fig. 31):

'When the larva approaches a rootlet with the object of affixing itself, which it does tail-foremost, the first movement which can be observed is that the strong bristles on the ventral valve are waved about, and their sharp tips inserted into the spot chosen. Doubtless, as suggested by Ingram and Macfie, these bristles are sensory, and may 'transmit a stimulus enjoining the larvae to attach themselves'. The tips of the ventral valves are then brought into contact with the root, and the three pairs of hooks, which are normally inside the tips of the valves, are everted. These hooks are directed posteriorly, the points of the stout bristles anteriorly, so that between them they can obviously afford the larva a tolerably firm preliminary 'foothold'. The hooks, it should be said, act quite independently of the other internal structures.

The larva then commences a series of violent jerks; the body, at first held in a curved position, is suddenly straightened out (without being lengthened), and at the same moment the anal segment is bent rapidly downwards. The first position is then more slowly resumed, and after a brief moment of rest the movement is repeated; this continues until the tip of the air-tube is firmly embedded in the grass root.'

Other examples of larvae which obtain oxygen from the roots of aquatic plants are those of an eristaline fly, *Chrysogaster hirtella* Loew, and an ephydrid, *Notiphila riparia* Mg., described and figured by Varley (1937). Both these larvae have their postabdominal spiracles transformed into perforating organs which they thrust into the roots of the grass *Glyceria aquatica* Sm. to reach the air spaces and obtain the necessary supply of oxygen.

I have had the opportunity of examining personally several aquatic larvae with postabdominal spiracles adapted as perforating organs. The larva of one of these species, *Hydrellia modesta*, has been described already (Keilin, 1915), the larvae of the other three species which obtain their air from the *Glyceria* roots include the larvae of an eristaline, probably a species of *Chrysogaster* (Fig. 32 A), of a tipulid, *Erioptera* sp. (B) and of an ephydrid, *Notiphila* sp. (C). The larvae with perforating spiracles provide a series of very interesting examples of convergence among families which are phylogenetically widely separated.

(4) *Perispiracular pneumatic sacs.* While the larvae of certain aquatic stratiomyids are provided with a more or less developed postabdominal spiracular siphon, those which live in a semifluid or even viscous medium of decomposed organic matter are completely devoid of such a siphon. The posterior end of their body is truncated and the postabdominal spiracles are found within a pneumatic sac. This was shown by de Meijere (1916) in the larva of *Pachygaster minutissima* Zett. and by Irwin-Smith (1923) for the larva of *Metopina rubiceps* Macquart.

It can also be clearly seen in the larvae of *Microchrysa polita* Lin. (Fig. 33 A–E). The larva is amphipneustic (A), with the prothoracic spiracles small and composed of two papillae only (B). The metathoracic and the first seven abdominal spiracles are not functional, being completely obliterated (C). The postabdominal spiracles (E, S_8) are present within a pneumatic sac (*p.s.*) which opens outside by means of a transverse slit (*Sl.*) surrounded by a sclerotized ring (*s.r.*). A number of dilatory muscles (*M*), which are found stretched between the pneumatic sac and body walls, control the admission of air into the sac. The spiracles (S_8) are circular and provided with a central scar-plug (*Sc.p.*) surrounded by radiating spiracular clefts. The larvae of stratiomyids which are provided with a pneumatic sac are usually very sluggish and can live by having only an intermittent contact with air.

(5) *Certain modifications of the tracheal system.* The main modification of the tracheal system found in certain oligopneustic aquatic larvae consists in excessive development of the tracheal trunks as in the case of eristaline larvae. In the larvae of *Ptychoptera paludosa* Mg., according to Miall (1895): 'In the centre of most of the segments the tracheae become greatly enlarged, the intervening portions, from which many branches are given off, being comparatively narrow. Each tube, therefore, resembles a row of bladders connected by small necks. A cross-section shows that the tubes are not cylindrical, but flattened, and that, while the lower surface is stiffened

by the usual parallel thickenings, the upper surface is thrown into two deep, longitudinal furrows....' The local enlargements or swellings of the tracheal trunks and the formation of a real air-sac can be seen in the larvae of certain mosquitoes such as *Orthopodomyia pulchripalpis* (Fig. 34 A) and *Taeniorhynchus richiardii* (Figs. 30 A, 34 B). (See Wesenberg-Lund, 1918; Marshall & Staley, 1933; and Marshall, 1938.)

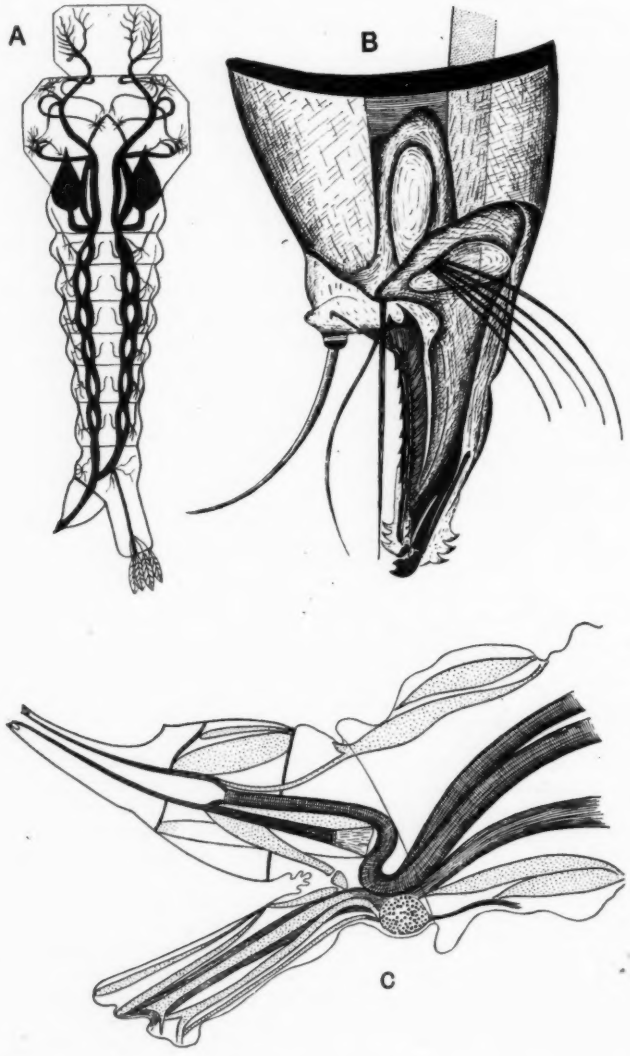


Fig. 30. *Taeniorhynchus richiardii* (Ficalbi) (after C. Wesenberg-Lund, 1918). A, tracheal system of the larva; B, postabdominal respiratory siphon of the larva seen sidewise; C, sagittal section through the posterior portion of the body.

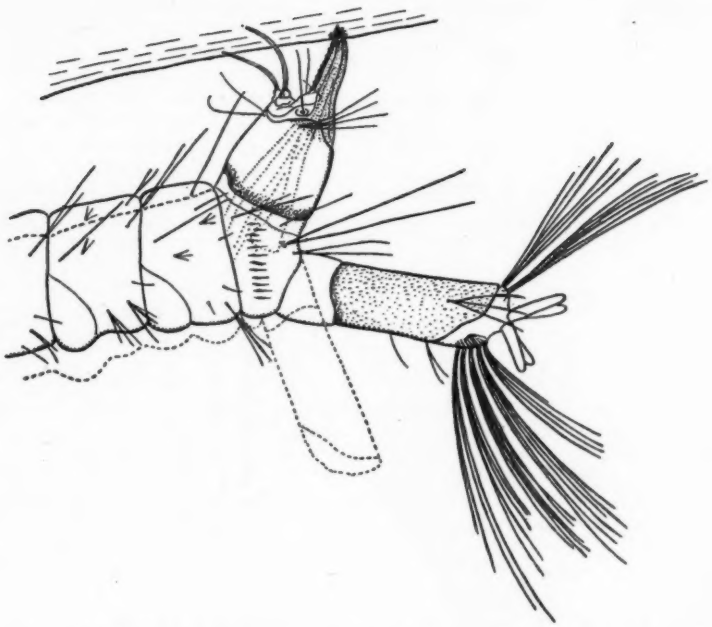


Fig. 31. *Taeniorhynchus richiardii*; posterior segments of the larva showing the mode of attachment to plants by means of perforating postabdominal spiracles (after Edwards from Marshall, 1938) (diagrammatic).

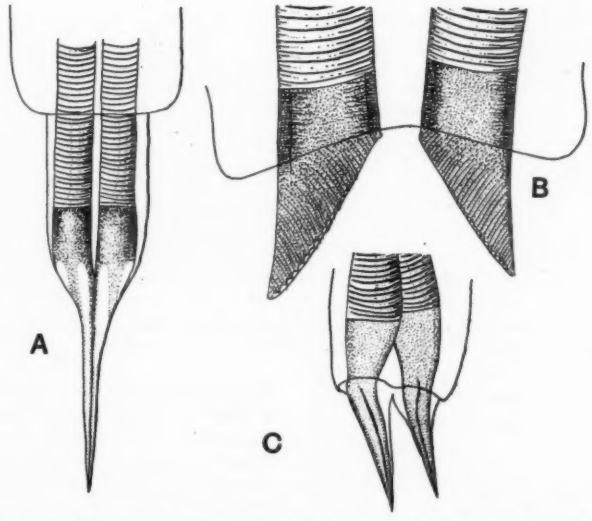


Fig. 32. Posterior portions of the larvae showing the postabdominal spiracles transformed into perforating organs in order to obtain air from submerged plants. A, *Chrysogaster* sp. (Eristaline); B, *Erioptera* sp. (Tipulidae); C, *Notiphila* sp. (Ephydridae). A and C × 85, B × 260.

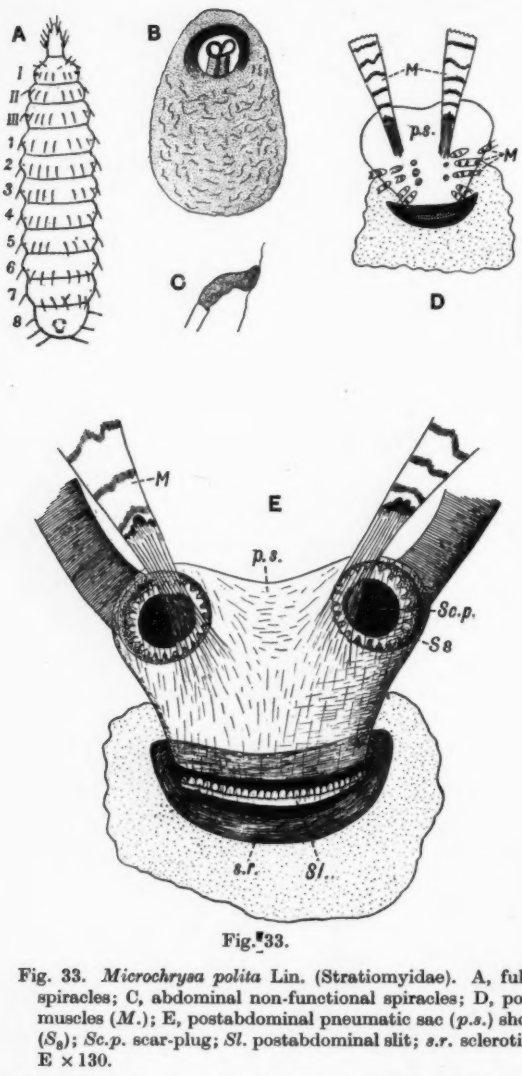


Fig. 33.

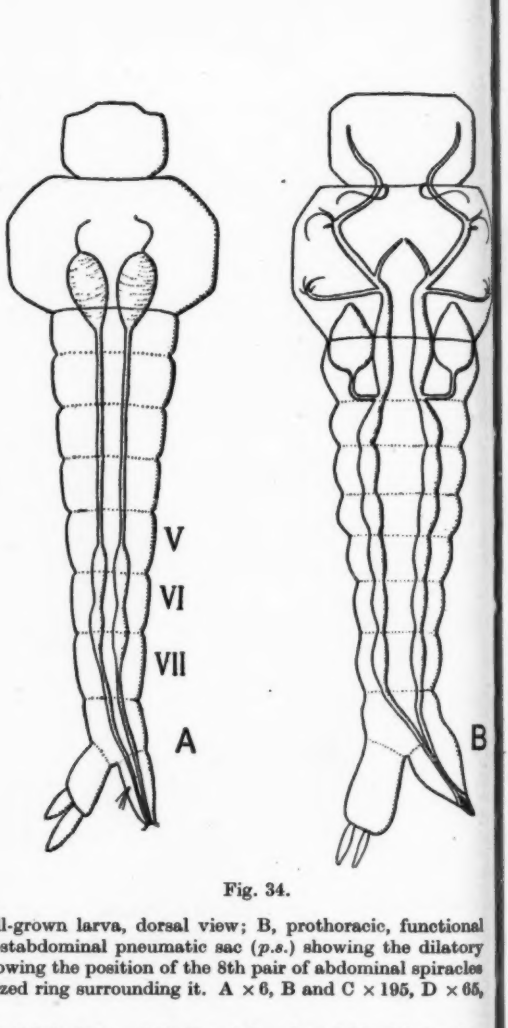


Fig. 34.

Fig. 33. *Microchrysa polita* Lin. (Stratiomyidae). A, full-grown larva, dorsal view; B, prothoracic, functional spiracles; C, abdominal non-functional spiracles; D, postabdominal pneumatic sac (*p.s.*) showing the dilatory muscles (*M.*); E, postabdominal pneumatic sac (*p.s.*) showing the position of the 8th pair of abdominal spiracles (S_8); *Sc.p.* scar-plug; *Sl.* postabdominal slit; *s.r.* sclerotized ring surrounding it. A × 6, B and C × 195, D × 65, E × 130.

Fig. 34. Tracheal system of larvae of A, *Orthopodomyia pulchripalpis* and B, *Taeniorhynchus richiardii* showing local dilatations and air sacs (after Marshall, 1938, p. 260).

XV. RESPIRATORY ADAPTATIONS OF PARASITIC LARVAE

It is a remarkable fact that practically all species of holometabolic insects, the larvae of which live as internal parasites of animals, are confined to two large and one small order of insects, namely, Diptera, Hymenoptera and Strepsiptera. It is interesting to note that, while the hosts of parasitic Hymenoptera and Strepsiptera belong only to arthropods, the hosts of Diptera are found among a great variety of animal groups, such as worms, molluscs, Crustacea, arachnids, myriapods, insects, amphibia, reptiles, birds and mammals including man. The study of Diptera reveals, on the other hand, that the great majority of species with parasitic larvae belong to the Cyclorrhapha. Among Orthorrhapha the larvae with a true parasitic habit are very uncommon.

Thus in the very large sub-order of Nematocera parasitism is known only in a few chironomids such as *Symbiocladius* parasitic on the ephemerids (Claassen, 1922; and Codreanu, 1939). Among Brachycera the larvae of a small family of Cyrtidae are known as true parasites of spiders, while the larvae of certain Bombylidae and Nemestrinidae have been recorded as parasites of bees, wasps and some other insects. Very little is known, however, as to the relationship between these larvae and their hosts.

On the contrary the cyclorrhaphous Diptera contain a large number of species parasitic in a great variety of hosts. Among them we shall find the best examples illustrating the respiratory adaptation of parasitic larvae which I propose to examine.

(*a*) *Larvae of* Pollenia rudis *parasitic in earthworms*

The best example of dipterous larvae parasitic in oligochaetes is that of *Pollenia rudis* Fabr. parasitic in the common garden earthworm, *Allolobophora chlorotica* Sav. (see Keilin, 1915). *Pollenia rudis* is a very common and widely distributed calliphorine fly usually living in the open but hibernating in houses which are often invaded by large populations of this fly. They are known then to aggregate into clusters, hence the name of 'cluster fly' given to it in America. The female of *Pollenia rudis* deposits its eggs, usually during September or October, in the soil of gardens or open fields. Small 1st stage larvae soon hatch from these eggs and, on reaching the earthworms (*Allolobophora chlorotica*) penetrate, probably through the male genital opening, into their coelomic cavity between segments 9 and 16, but more often directly into the sperm sacs. The larva gradually passes into a kind of hibernal immobility which lasts for about 7–8 months. During this period the small 1 mm. long larva does not feed, it remains almost motionless and becomes surrounded by numerous concentric layers of phagocytic cells of the earthworm. These larvae can be easily seen by transparency in a worm compressed between two slides and examined with a strong source of light and low magnification (Fig. 35 A, *L*). In spring when the temperature of the soil reaches about 20° C., the larva begins to move; it breaks the phagocytic cyst, leaves the sperm sacs, and while free in the coelomic cavity of the earthworm it migrates from the genital segments towards the anterior end of the host. In the anterior region of the worm the larva takes a position parallel to the host and moves with its posterior end directed forward. The larva finally reaches the dorsal lip or prostomium (Fig. 35 B) of the earthworm and perforates it from inside by rubbing against it with its posterior end. Through the aperture in the prostomium the larva protrudes the posterior end of the body, thus bringing the postabdominal spiracles in contact with the outer air (Fig. 35 D). It now begins to feed very actively, and 6 days after perforation of the prostomium the larva undergoes a moult thus passing into the second larval stage during which it grows considerably and destroys the partition which separates it from the pharynx of the host (Fig. 35 C). The second stage lasts only about 9–10 days. The larva moults again and passes into the third larval stage during which it devours the tissues of the host keeping all the time its posterior end with spiracles protruding from the worm (Fig. 35 E). The anterior end of the latter becomes distended, heavy, inflamed and gradually disintegrates, leaving the larva more and more exposed. Finally, the larva is seen to be attached by its anterior end to the remaining portion of the worm which is still alive (Fig. 35 F). In this position the larva devours almost completely the remaining segments of the worm, and then buries itself deeper into the soil, forming a puparium from which, 30–45 days later, emerges the adult fly.

The larval life of *Pollenia rudis* can be divided into four distinct periods: (1) short active period of the first stage larva before the invasion of the host, (2) long inactive periods of life in the coelomic cavity of genital segments, (3) short period of migration towards the prostomium, (4) perforation of the prostomium, protrusion of posterior end of the larva through the aperture and very active feeding and growth accompanied by two moults.

The respiratory system of the first stage larva is metapneustic and the tracheae, filled with air, are functional during the active period previous to the invasion of the host. During 7 months of intracoelomic life the larva does not grow, and although its tracheae are filled with air, the respiratory activity is very low, the larva living upon oxygen dissolved in the surrounding medium. This inactivity is undoubtedly due to the low temperature. In fact, at any time during this long period of hibernation the larvae can be aroused from this lethargic state simply by raising the surrounding temperature to about 20° C. The larva then becomes active, migrates towards the prostomium, and perforates it in order to bring its postabdominal spiracles in contact with air. While the larva has to depend upon the oxygen dissolved in the medium it does not take any food. It is only when its respiratory requirements are satisfied that active feeding and growth of the larva take place. The larva then moults and passes into the second stage which is amphipneustic, with the anterior spiracles composed of four small papillae, and the postabdominal pair showing two characteristic slits. After a second moult, the third stage larva remains amphipneustic but with a highly developed tracheo-spiracular system. The prothoracic spiracles, much larger now, are composed of four papillae, while the postabdominal spiracles show the three characteristic oval peritremes converging

towards the external scar. Throughout the active period of their parasitic life cycle the postabdominal spiracles are kept in communication with the open air.

emerges from the egg bores its way into the kidney (*k.*) of the host where it lies with its posterior end, bearing the spiracles, protruding into the pulmonary or mantle cavity of the snail. During this stage the

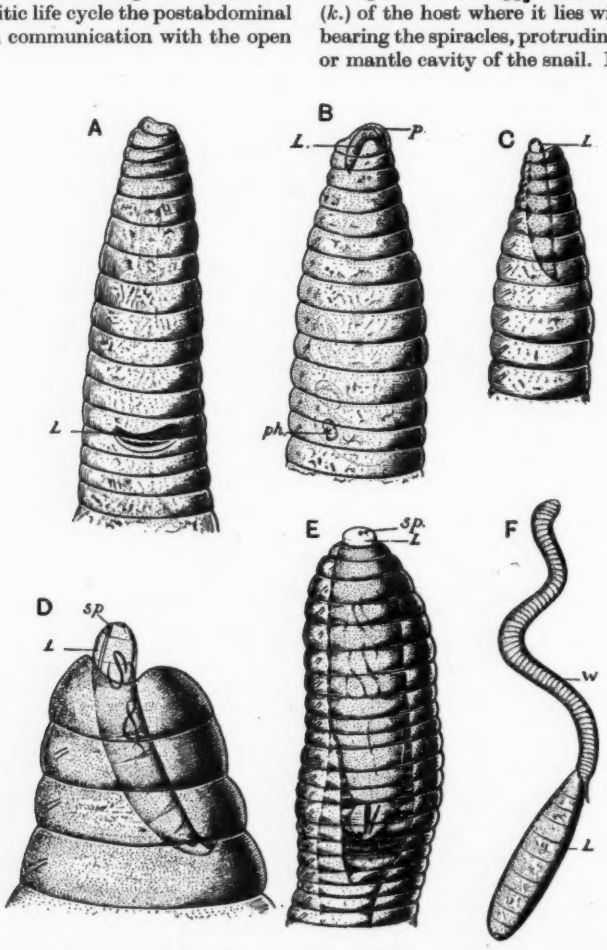


Fig. 35. *Pollenia rudis* Fabr. Parasitic in *Allolobophora chlorotica* Sav. A, anterior portion of earthworm (*All. chlorotica*) showing by transparency a small first stage larva (*L.*) lying in the body cavity between segments 14 and 15 (November); B, earthworm with one larva (*L.*) of *Pollenia* in prostomium (*p.*) and one phagocytized (*ph.*) within the sperm sac (May); C, earthworm with the second stage larva (*L.*) of *Pollenia* protruding its postabdominal spiracles outside host's body; D, first stage larva (*L.*) of *Pollenia* protruding its posterior segments through the aperture in the prostomium of the earthworm; E, earthworm with third stage larva (*L.*) of *Pollenia* showing postabdominal spiracles (*sp.*) exposed to air: reconstructed from two specimens; F, full-grown larva (*L.*) of *Pollenia* attached to remains of earthworm (*W.*). A, B and C × 7, D × 25, E × 8.

(*b*) *Larvae of* Melinda cognata *parasitic in snails*

The only well-known case of a dipterous larva parasitic in molluscs is that of a calliphorine fly, *Melinda cognata* Meig., parasitic in *Helicella* (*Heliomanes*) *virgata* Da Costa (see Keilin, 1919). The female of this fly lays one or two eggs (*ov.*) at a time into the pulmonary or mantle cavity (*m.c.*) of the snail (Fig. 36 A). The small metapneustic larva (*L.*) which

host shows no signs of distress. The larva moults and passes into the second stage which is amphipneustic. Now it grows more rapidly, destroys the kidney and appears within the mantle cavity of the host (Fig. 36 B, *L*) with its postabdominal spiracles directed towards the pneumostome. The larva undergoes the second moult and passes into the third stage which remains amphipneustic but with the tracheo-

spiracular system much more developed. The larva feeds very actively, grows rapidly and attacks the liver of the host. The snail no longer moves and retracts its body within the shell (Fig. 36 C). The larva occupies the whole of the pulmonary cavity and protrudes its posterior end through the pneumostome, thus bringing its postabdominal spiracles (*sp.*) in contact with the external air. All the organs of the snail are attacked in turn. The liver is destroyed, the alimentary canal is perforated and the snail ultimately dies before the larva is full grown. The larva finally becomes saprophagous, living upon

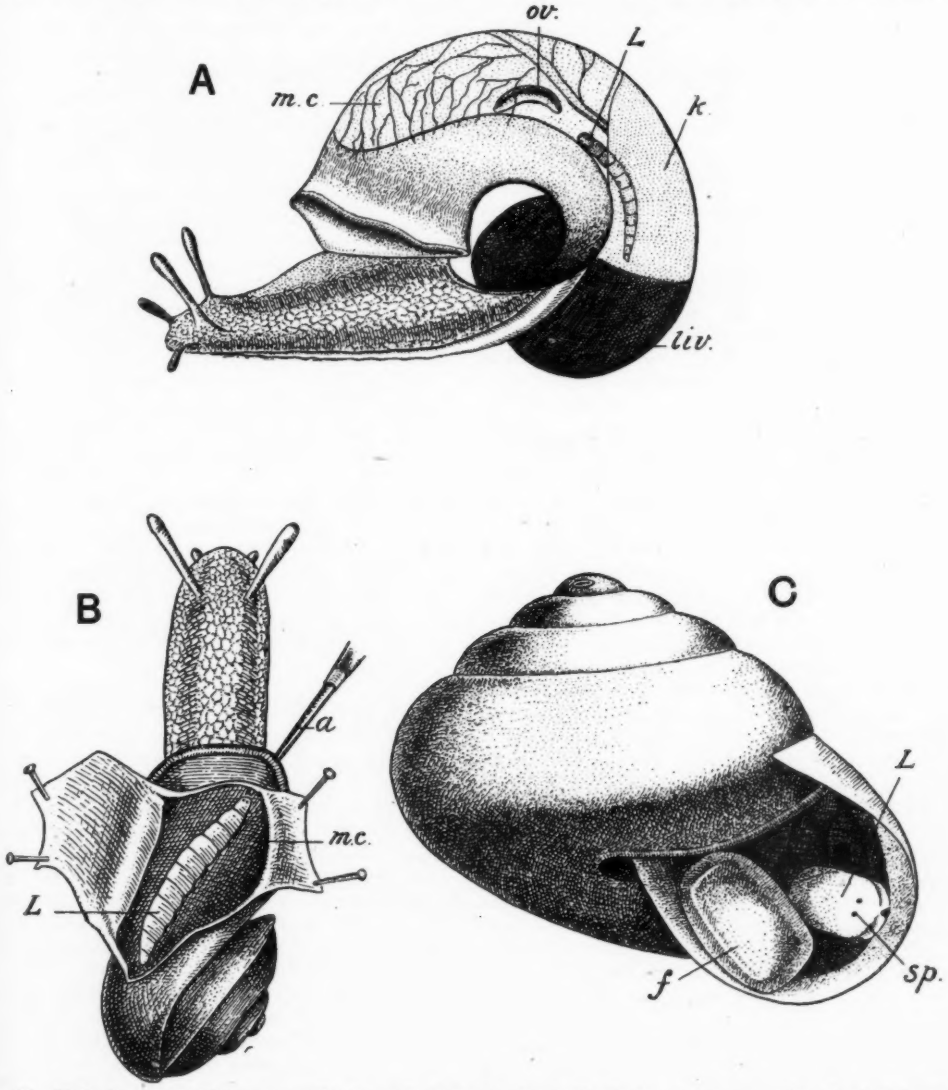


Fig. 36. *Melinda cognata* Meigen parasitic in snail, *Helicella virgata* Da Costa. A, young snail extracted from its shell and showing by transparency in its mantle cavity (*m.c.*) an egg (*ov.*) and in the kidney (*k.*) the first stage larva (*L.*) of *M. cognata*. B, snail, with anterior part of its body removed from the shell and with opened mantle cavity (*m.c.*) showing a large second stage larva (*L.*) of *Melinda*; the dissecting needle *a* points the position of pneumostome of the host; C, snail retracted within its shell and showing posterior end with spiracle (*sp.*) of third stage larva of *Melinda* protruding from pneumostome of host; *f.* foot of snail.

the decomposed remains of the snail. As the food supply is exhausted, the larva works its way into the upper whorl of the shell which it occupies completely. During the whole of the saprophagous period, the posterior spiracles of the larva remain in communication with the external air. The larva, when full grown, leaves the shell, penetrates into the earth, and by the next day turns into a pupa from which about 14 days later emerges the adult fly.

All this clearly shows that the larva of *Melinda* throughout its life cycle keeps the postabdominal pair of its spiracles in communication with the air.

(c) *Larvae parasitic in terrestrial arthropods*

(1) *Larvae with spiracles attached to respiratory apertures and surrounded by chitinous envelopes*

It is among the arthropods that one finds the largest number of hosts harbouring parasitic dipterous larvae. The best example for the study of the respiratory adaptation of larvae belonging to this group is that of *Thrixion halidayanum* Rond., the life history of which was exhaustively dealt with in the classical monographic study by Pantel (1898). The female of *Thrixion* fixes its eggs upon the body of *Leptynia hispanica*. When the larva of the parasite is completely formed it perforates the egg shell as well as the integument of the host supporting it and penetrates into its body cavity. For a short period the larva of *Thrixion* remains free in the perivisceral cavity of its host and undergoes the first moult. The second stage larva, by means of the hooklets surrounding the postabdominal spiracles, perforates the body wall of the host from inside and fixes its spiracles into this newly formed aperture. The host shows a peculiar inflammatory reaction around this aperture: the hypoderm proliferates and secretes a thin chitinous sac which surrounds almost completely the parasitic larva. The proximal portion of this sac becomes strongly sclerotized forming a funnel-like structure or a siphon within which is engaged the posterior end of the larva. In this position the larva feeds and grows rapidly, undergoes a second moult and remains throughout its third stage. When full grown the larva leaves the host through this aperture and pupates in the soil.

The life cycle of *Thrixion halidayanum* can be characterized as follows: formation of the primary aperture through which the larva invades the host, a period of free life within the body cavity of the host, and formation of a second aperture from within in order to bring the spiracles in contact with the outside air. This type of life cycle is very common among dipterous larvae parasitic in arthropods. There are, however, several interesting variations of this type investigated by Pantel (1909) and other workers and which can be summarized as follows:

(i) *The respiratory aperture* through which the parasitic larva protrudes its postabdominal spiracles may be either a *primary aperture*, being the aperture of invasion of the host as it is in the case of *Ptychomyia selecta* Meig., *Echinomyia fera* L., *Bigonichaeta setipennis* Fall. and others or a *secondary aperture* made from inside as in the case of *Thrixion halidayanum* and several other tachinids. The secondary aperture may have different localizations, namely, (α) the integument of the host (Fig. 37 A) as it is in *Panzeria rudis* Fall., *Thrixion halidayanum*, *Meigenia floralis* Meig., *Ceromasia rufipes* B.B., *Carcelia cheloniae* Rond., (β) the tracheae of the host, (Fig. 37 C) as it is in *Hyria tibialis* L., *Blepharidea* (Fig. 37 C) *vulgaris* Fall. and *Gymnosoma rotundatum* L., or (γ) the spiracles of the host as it is found in *Compsilura concinnata* Meig.

In all these cases the host reacts in the same way, by surrounding the body of the parasite with a chitinous envelope (*ch.e.*), the proximal portion of which is hardened to form a respiratory funnel (Fig. 37 A, C, *fn.*).

(ii) *Localization of parasitic larvae prior to their fixation.* The young parasitic larva which does not remain fixed to the aperture of invasion (primary fixation) may remain for a short period of time either completely free in the body cavity of the host as in the case of *Thrixion halidayanum*, *Meigenia floralis*, *Gymnosoma rotundatum*, etc., or it may invade temporarily different organs and tissues of the host. Thus the young larvae of *Sturmia pupiphaga* invade the muscle fibres, those of *Grossocosmia sericaria* invade the nerve ganglia, whilst others invade the fat-body cells, genital glands, or the silk-gland cells of their host.

(2) *Larvae attached to tracheae of host and devoid of chitinous envelope*

To this group belong the larvae of Conopidae parasitic in Hymenoptera (de Meijere, 1904). The parasitic larvae are fastened to the large or vesicular tracheae of the host by means of chitinous structures connected with their postabdominal spiracles. Although no distinct aperture in the host trachea is visible, the parasitic larva cannot be detached from the trachea without tearing the latter. Unlike the cases discussed above, the tracheal epithelium of the host parasitized by the larvae of Conopidae does not react to form the chitinous sheath surrounding the parasitic larva. The gas exchange between the latter and the host takes place apparently through a very thin chitinous membrane of the main trachea of the host.

(3) *Larvae with spiracles perforating integument of host but devoid of chitinous envelope*

To this group belong the larvae of *Cryptochaetum* parasitic in Coccidae. A complete account of the

life history, parasitic adaptations and larval morphology of several species of *Cryptochaetum* can be found in a series of interesting papers by Thorpe (1930–31, 1934, 1941). The respiratory adaptation of these larvae is clearly shown by *Cryptochaetum striatum* parasitic in *Aspidoproctus maximus* (see Thorpe, 1941). The eggs of *Cryptochaetum* are laid in about five times longer than the body of the larva (Fig. 38 F). Both the first and the second stage larvae are found free in the blood of the peripheral region of the host. The third stage larva is marked by a very great development of the caudal processes which are now about ten times longer than the body of the parasite (Fig. 38 A). They are packed with

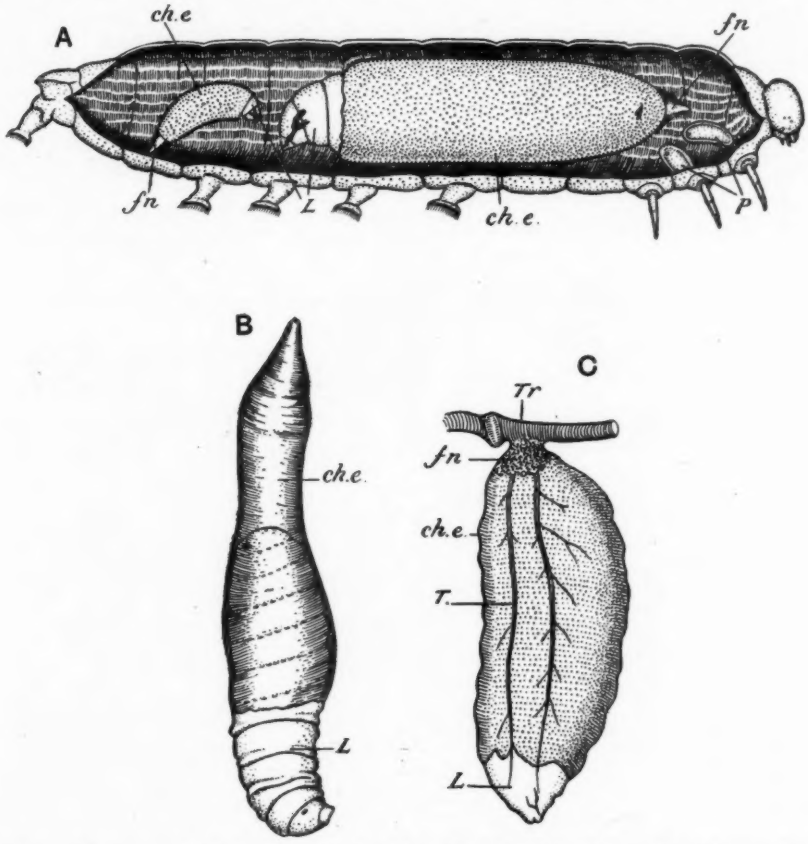


Fig. 37. Dipterous larvae parasitic in insects. A, caterpillar of *Panolis piniperda* parasitized by two first stage (*p.*), one second stage and one full-grown larva of *Panzeria rudis* Fall.; B, full-grown larva of *Panzeria* partly enclosed within its sheath detached from host's tegument (Figs. A and B after Prell, 1915); C, larva of *Hyria tibialis* (L.) surrounded by a chitinous envelope originating from trachea of its host—*Vanessa*. *ch.e.* chitinous envelope or sheath; *fn.* respiratory funnel; *L.* parasitic larva; *T.* tracheal trunks of parasitic larva; *Tr.* hosts tracheae (after Pantel, 1909). C × 17.

the body cavity of the half-grown scale insect. The first stage larva of the parasite is devoid of mouth-parts, segmentation, tracheae and spiracles. It is, however, provided with a pair of long caudal processes containing blood. The second stage larva is normally segmented, has normally developed mouth parts and well-developed tracheae with branches penetrating far into the caudal processes, which are tracheal filaments which may extend to about two-thirds the length of the processes (Fig. 38 B). The larva is now amphipneustic and provided with two pairs of well-developed spiracles. The prothoracic spiracles which are invaginated within a furrow are composed of five finger-like processes originating from a common short and wide felt chamber (Fig. 38 C). The postabdominal spiracles protrude from

the surface of the last abdominal segment, they are bent and pointed and have the very characteristic hook-like appearance of a perforating organ (Fig. 38 D). The young third stage larvae still remain free in the body cavity of their host, while the older larvae are found in the visceral chamber with their post-anterior ones (Fig. 38 E). The prothoracic spiracles are now evaginated and thrust through the same membrane as the postabdominal ones. This is followed by a hardening and darkening of the cuticle which marks the formation of the puparium. The adult insect emerges into the egg pouch of the host

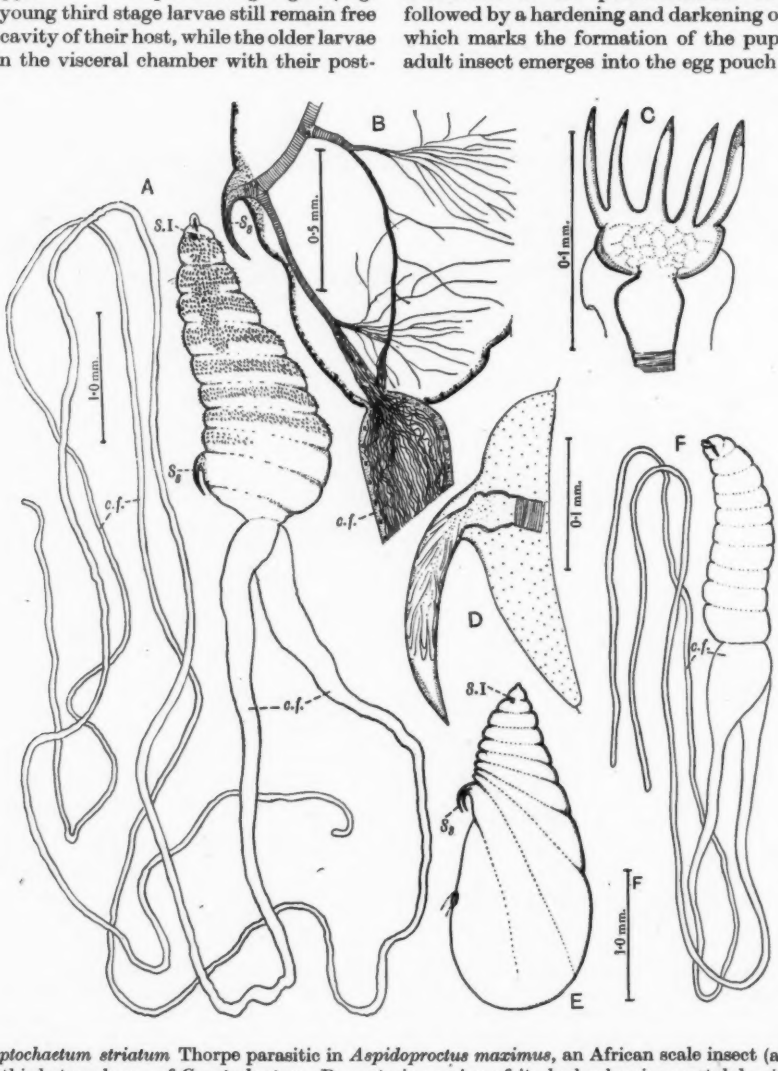


Fig. 38. *Cryptochaetum striatum* Thorpe parasitic in *Aspidoproctus maximus*, an African scale insect (after Thorpe, 1941). A, third stage larva of *Cryptochaetum*; B, posterior region of its body showing postabdominal spiracles (S_8) and tracheal supply of caudal or filamental process (*c.f.*); C, prothoracic spiracles; D, postabdominal spiracles; E, change in form of third stage larva prior to pupation; F, second stage larva; *S.I*, prothoracic spiracles.

abdominal spiracles thrust through the wall separating it from the egg chamber, thus bringing them into contact with the outside air. When the larva is ready to pupate the dorsal portion of segments 4–8 undergo a gradual contraction bringing the posterior spiracles forward until they come to lie close to the by breaking off the puparial lid and the thin membrane of the pouch.

The postabdominal processes in this larva especially during the first, the second and the early third stages act as tracheal gills. The spiracles begin to be functional only during the latter part of the

third stage when they perforate the membrane surrounding the egg pouch and come in contact with the external air. The larval spiracles of the puparium provide also the gas exchange between the air space surrounding the pupa and that of the egg pouch which opens outside.

(4) *Larvae which contract no definite respiratory relationship with hosts*

These larvae are found lying free within the body cavity of their host and, although they are provided with a well-developed tracheo-spiracular system, they never fasten their spiracles to, or thrust them through the body or tracheal wall of their host. The postabdominal spiracles in these larvae often protrude in the form of hardened dark tubercles bearing numerous spiracular papillae. This is the case with *Fortisia foeda* parasitic in *Lithobius* (Thompson, 1915) and the larvae of pipunculids parasitic in *Typhlocyba* (Keilin & Thompson, 1915). It is conceivable that these larvae by means of their hardened spiracles damage small tracheae or intersegmental membranes of their host, thus establishing an intermittent contact with the air bubbles appearing in the body cavity of the host. To this category belong also the larvae of Sarcophaginae and Miltogramminae parasitic in different Orthoptera observed by Künckel d'Herculais (1894) and Pantel (1909).

(*d*) *Larvae parasitic in aquatic arthropods*

Three interesting cases are known of larvae parasitic in aquatic insects:

(i) Larvae of a tachinid (*Siphona cristata* Fabr.) found by Roubaud (1906) as parasitic in the larvae of *Tipula gigantea* Schr. The host larvae live under the stones in streams bringing only their postabdominal spiracles intermittently in contact with the fresh air. The parasitic larva thrusts its postabdominal spiracles through an aperture made in the main tracheal trunk of the host which reacts in the usual way by forming a chitinous sheath surrounding the parasite. This case of respiratory adaptation belongs therefore to the same category as that of *Hyria tibialis* (cf. p. 45 and Fig. 37 C).

(ii) Larvae of *Ginglymyia acrirostris* Towns. found by Lloyd (1919) as parasitic in an aquatic lepidopterous larva *Elophila fulicalis*. The larvae of the host live in swift-running waters under a thin and irregular web of silk. It is provided with gills. At the time of pupation the irregular web is replaced by a thick roof-like silk covering with semicircular openings at the ends facing up and down stream. Although the pupa is beneath the surface of the water, its back bearing the spiracles is surrounded by air. A full-grown larva of *Ginglymyia* distends the body of its host (Fig. 39 B) and thrusts its strong postabdominal spiracles through the suture sepa-

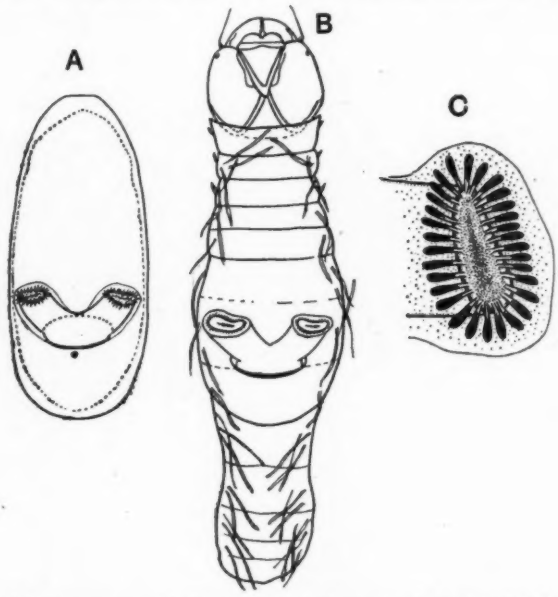


Fig. 39. *Ginglymia acrirostris* Towns. parasitic in aquatic Lepidopterous larva *Elophila fulicalis* (after J. T. Lloyd, 1919). A, pupa of *Ginglymyia*; B, larva of *Elophila* with postabdominal spiracular siphon of *Ginglymyia* protruding outside the host's body; C, postabdominal spiracle of parasitic larva.

rating the fourth and fifth abdominal segments. The two spiracles are supported by a V-like siphon protruding from the dorsal side of the postabdominal segment of the parasite. The majority of parasitized specimens shows only the remains of *Elophila* larva covering a single puparium of the parasite. The latter (Fig. 39 A), like a full-grown larva, shows the characteristic pair of postabdominal spiracles. These spiracles are in contact with air bubbles imprisoned under the roof-like silk which covers the host in its prepupal stages.

(iii) *Chironomid larva ectoparasitic on ephemerid.* In 1922 Claassen described a chironomid larva *Symbiocladius equitans* parasitic upon a may-fly nymph (*Rhitrogena*). His observations were confirmed by other workers and especially by Codreanu (1939), who published an extensive monographic study on *Symbiocladius rhithrogenae* (Zavr.) ectoparasitic upon torrential ephemerids. The first stage larva of *Symbiocladius* settles under the mesothoracic wing cases of the nymphal host where it weaves a silky sac which surrounds it completely except the mouthparts which are attached to the host. The parasite makes a small aperture in the integument of the host and feeds mainly upon its blood and leucocytes.

In this position the larva grows (Fig. 40 A), passes through four stages separated by three moults and turns into a pupa (Fig. 40 B), from which emerges the adult chironomid. Although the larva is confined to a fixed position where its body is curved and shows no movements, its respiratory requirement is satisfied owing (1) to a well-developed tracheal system, (2) the respiratory movements of the tracheal gills of the host and (3) the highly aerated water which is inhabited by the host.

(e) *Larvae parasitic in Amphibia, reptiles and birds*

The larvae of *Lucilia bufonivora* which attack toads, oviposit according to Portchinsky (1892) upon the skin of the host, and the young larvae on hatching from the eggs penetrate into their mouth and nasal cavities. Throughout their life while they feed on the host's tissue, they keep their postabdominal spiracles exposed to air.

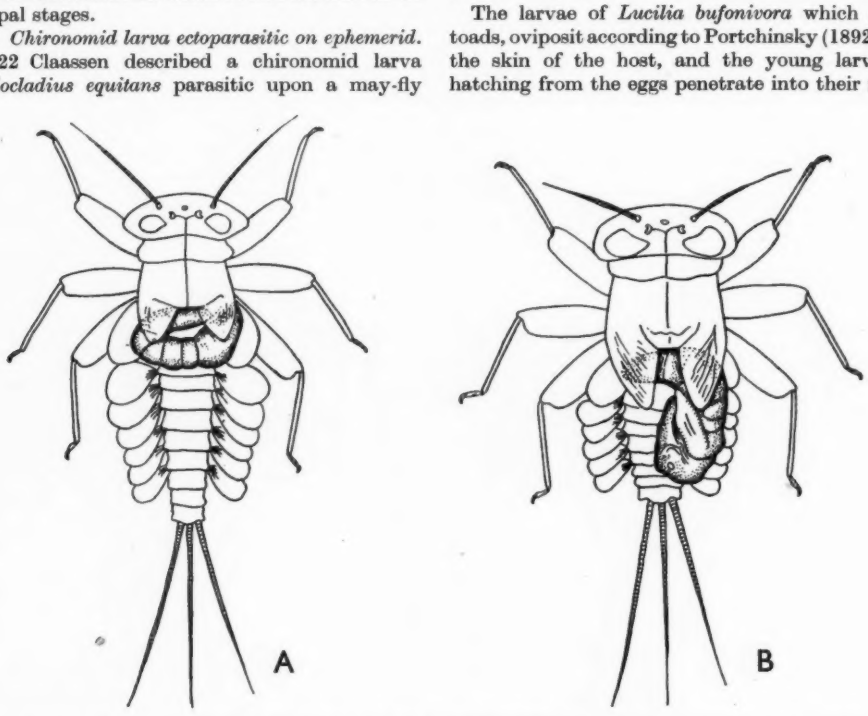


Fig. 40. Larva of chironomid—*Symbiocladius equitans* ectoparasitic upon may-fly nymph (*Rhitrogena* (after Claassen, 1922)). A, larva of *Symbiocladius* in the same position; B, pupa of *Symbiocladius* under wing pad of may-fly.

A few cases of sarcophagine larvae which are known to attack reptiles produce only external myiasis resembling that of *Lucilia bufonivora*.

Dipterous larvae attacking birds may be either blood sucking like *Protocalliphora azurea* Fall. or *Passeromyia heterochaeta* Villen., or they may produce subcutaneous tumours where they live and grow as in the case of *Mydaea anomala* Jaenn. and of a few other anthomyids (Nielsen, 1911, 1913). The tumour is usually of a large size and has at its apex a small round aperture which enables the larvae to

bring its postabdominal spiracles in communication with air.

(*f*) *Larvae parasitic in mammals*

The dipterous larvae which live upon mammals are either intermittent bloodsuckers like those of *Auchmeromyia luteola* (Fab.) and *A. chaerophaga* (Roubaud) or real parasites like those of *Cordylobia anthropophaga* (E. Blanchard) and different Oestridae. The study of three examples, namely, *Dermatobia*, *Hypoderma* and *Gastrophilus*, will give a general idea of the respiratory adaptations of larvae parasitic in mammals.

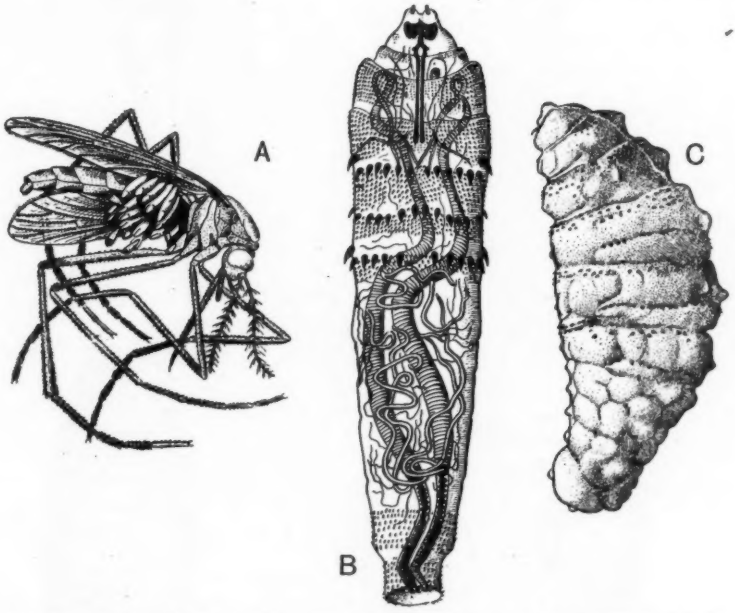


Fig. 41. *Dermatobia hominis* (Linn.). A, *Janthinosoma lutzi* ♀ carrying eggs of *Dermatobia*, × 5·5; B, first stage larva of *Dermatobia* emerged from one of these eggs, showing well-developed tracheal system, × 100; C, full-grown larva of *Dermatobia* (A and B after Surcouf, 1913; C after Sambon, 1915).

(1) *Dermatobia hominis* (Linn. 1781)*

The larvae of this fly cause the well-known cutaneous myiasis in man and different mammals throughout tropical America. It is interesting to note that among all the parasitic flies, this species is exceptional in having an intermediate host. The female of *Dermatobia* fixes its egg mass upon the body of a mosquito, *Janthinosoma lutzi* (Fig. 41 A), or an anthomyid fly, *Anthomyia heydenii*. When these intermediate hosts feed upon mammals, including man, the small larvae of *Dermatobia* (Fig. 41 B) emerge from the eggs and penetrate under the skin of their final host, where they grow and produce painful tumours. The latter show at their apex a small aperture by means of which the larva of *Dermatobia* puts its postabdominal spiracles in communication with air.* The respiratory system of a full-grown larva is amphipneustic with the postabdominal spiracles showing the three characteristic slits. The prothoracic spiracles discovered by Umaña (1915) are always retracted in the larva into a deep furrow. They evaginate only in the pupa (Fig. 42 D, *e.sp.*, E) forming thick, short projections giving rise to a large number of small filaments. In the larva these spiracles can be seen by transparency (Fig. 42 A, *p.sp.*) or on dissection of its anterior region (Fig. 42 B). A longitudinal section of such a spiracle (C) clearly shows the distribution of the spiracular

* For a full account of the life history of this fly and an exhaustive literature dealing with this subject the reader is referred to a very interesting paper by Sambon (1915). See also Newstead and Potts (1925).

* It is interesting to note that the respiratory adaptation of this larva was unwittingly used by the natives of different countries for getting rid of the infection. Thus according to Smith (1892) the Brazilians often tie a piece of fresh pork fat over the tumour with the result that the maggot leaves the tumour and burrows into the pork, while 'some of the ingenious Gringoes of Guatemala hit on the idea of placing a postage stamp over the hole' of the tumour (Folkes, 1897, quoted by Sambon, 1915, p. 146).

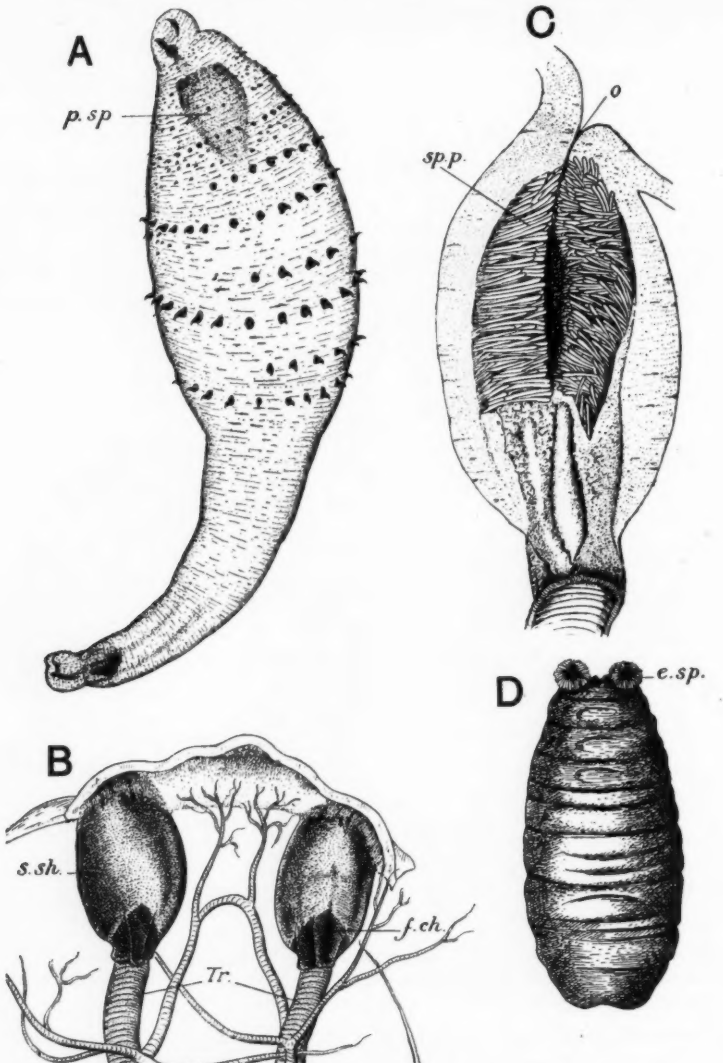


Fig. 42. *Dermatobia hominis*. A, young third stage larva seen from the side and showing by transparency the prothoracic spiracles (*p.sp.*); B, anterior portion of tracheo-spiracular system seen in a dissected larva (*f.ch.* felt chamber; *s.sh.* spiracular sheath; *Tr.* tracheal trunks); C, longitudinal section of prothoracic spiracle showing numerous spiracular papillae (*sp.p.*) (*o.* opening of spiracular furrow); D, puparium showing evaginated prothoracic spiracles (*e.sp.*); E, the latter more highly magnified (D and E after Umaña, 1915).

papillae (*sp.p.*) or filaments and the opening (*o.*) through which the evagination takes place. In the first and second stages the larva is metapneustic, becoming amphipneustic only in the third stage.

(2) *Hypoderma bovis* De Geer and *lineatum* (de Villers)

The occurrence of tumours or warbles in the backs of cattle from which emerge large grubs has been known for many centuries. In medieval England a cow with its hide perforated by warbles was said to be elf-shot. Vallisnieri was the first to obtain in 1710 the adult fly bred from a warble maggot which was described and illustrated in his *Opere* published by his son in 1733. Much work has been done on these insects since Vallisnieri, but it was only between 1908 and 1920 that the main stages of their life history were unravelled by the careful and painstaking work of Carpenter (1908–20), Hadwen (1916) and their co-workers.*

The female of *Hypoderma* lays its eggs—1–14 at a time—on the hairs of cattle, usually those of the legs, but never in the region where the warbles ultimately appear. Although the oviposition inflicts no pain on the host, the mere presence of the fly causes excitement and unrest characteristic of 'gadding'. The oviposition is at its maximum in July, the fly being capable of laying 800 eggs in all. The egg hatches in 4–7 days, giving rise to a small spiny larva less than 1 mm. long, which is metapneustic and provided with a well-developed tracheal system. The larva crawls down the hair, bores into the skin through the hair follicle, and is completely lost to sight for several months. In September, October and especially November the larvae appear in the pharyngeal region of the wall of the oesophagus, lying in the connective tissue beneath the submucosa. These larvae are 6–14 mm. long, metapneustic, smooth and very transparent. They work their way towards the diaphragmatic end of the oesophagus, pass under the pleura across the diaphragm until they gain the cartilages of the ribs. They follow the vessels between the intercostal muscles and travel towards the dorsal integument often passing through the spinal canal between the periosteum and the dura mater. In February or March the larva reaches the spot for its final development, entering the hide from inside and begins to form a 'warble'. It undergoes a moult, pierces a hole to the exterior into which is thrust the postabdominal segment with its pair of spiracles, and in this position the larva feeds and grows actively. The full-grown larva emerges from the tumour through a wide aperture used for respiration, falls to earth, burrows into the ground, and forms a puparium from which in about 5 weeks emerges the adult fly. From July, the time of penetration into the skin of the host, until March when the warbles become perforated, namely, for at least 8 months, the larvae of *Hypoderma* have no direct communication with air. During this period the larvae wander about within the tissues of their host covering long distances. They grow from 1 mm. in length to about 14 mm., change their appearance and live upon the oxygen dissolved in surrounding tissues, the gas exchange taking place through the whole surface of their body. The larva depends obviously upon the same oxygen supply as the tissues of the host, and although during this period it grows in size, this increase is to some extent an apparent one, resembling more a dropsical condition than actual growth and differentiation of cells and tissues. In fact, the larva acquires the appearance of an elongated and completely transparent gelatinous sac. It is only after formation of the warble and its perforation, which enable the larva to bring its postabdominal spiracles in communication with air, that the larva begins to feed and to grow actively.*

Knowledge on the development of the respiratory system in *Hypoderma* larva is far from being complete considering that there is no agreement even on the number of larval stages separated by moults (see Laake, 1921). According to Walter (1922), who studied three larval stages, the postabdominal spiracles in a larva 14 mm. long are rudimentary and non-functional. It becomes functional only in a larva 18 mm. long. He found also certain peculiarities in the structure of the tracheae, namely, that the tracheae often break into bundles of numerous long tracheoles lying free in the perivisceral fluid of the larva.

(3) *Gastrophilus intestinalis* (De Geer)

Much work has been done on the life history and larval anatomy of different species of *Gastrophilus*. The description of the respiratory system of these larvae can be found in the papers by Enderlein (1899), Prenant (1900), Portier (1911) and more recently that by Dinulescu (1932), which also contains a complete historical account of the work

* All this work was well summarized by Warburton (1922).

* It is interesting to note that, as in *Dermatobia*, the respiratory adaptation of the *Hypoderma* larvae helped incidentally to discover one of the methods of killing the warble larvae. Miss Ormerod (1886) tells for instance how in her time in Ireland charms were used against the warbles: 'The "charmer" is generally an old woman. When she enters the stable of the sick cow, she calls for some butter or lard. After it has been placed before her, she prays for a time to some spirit. After the spirit of destruction is exorcised she takes the butter and gently covers the breathing aperture of the maggot and crosses it. The result of all this is that the maggots die, and fall, or are easily picked out, without causing the least pain.'

published on this subject. There are about six well-established species of *Gastrophilus* of which three, namely, *G. intestinalis*, *G. haemorrhoidalis* and *G. nasalis*, are cosmopolitan, while two, *pecorum* and *meridionalis*, are more localized. The life history of these parasites is shown typically enough by that of *G. intestinalis*. The female fixes its eggs to the hairs of the horse, usually those of the legs, breast and abdomen. In about 5–6 days the larvae are completely formed but they emerge only on contact with the tongue of the host and penetrate under its epithelial layer, forming a superficial gallery. They migrate towards the pharynx, undergo the first moult, leave their gallery and become attached to the mucosa of the pharynx, especially under the epiglottis. They stay in this position only for a short time and then migrate into the stomach where they are found attached by means of their mouth-hooks to the mucosa of the cardiac region. The larvae undergo a second moult, and pass into the third stage which is usually reached within 45–60 days after the first moult. The larva grows rapidly and full-grown larvae are found already between October and November. They remain, however, in a quiescent stage for at least 7–8 months, until May or June, and during this long period of quiescent life they feed very little and their respiratory activity is at its lowest. The larvae finally leave the alimentary tract, burrow into the soil or crevices in the ground and pupate. The adult fly emerges from the pupa within 20–30 days.

The respiratory system of the first stage larva is metapneustic, and during its migration under the epithelial layer of the tongue the respiratory requirements of the larva are well satisfied by the rich oxygen supply in the surrounding tissues and the possibility of breaking the gallery in order to bring the postabdominal spiracles in contact with air. After the first moult, the larva becomes amphipneustic, and during a short period of its life in the pharynx of the host the whole body of the larva is exposed to air. During the next 7–8 months of life within the stomach of the host the larva is immersed in a semifluid medium deficient in oxygen. It has to depend on an intermittent contact with air bubbles deriving from the air spaces of ingested food, or air swallowed with the food especially if a heavy infection with *Gastrophilus* larvae should predispose the host to a certain degree of aerophagy. The respiratory apparatus of *Gastrophilus* larvae is nevertheless highly developed and specialized for the aerobic mode of life under exceptionally unfavourable conditions. This is clearly shown by several important features of this system which enable the larva to overcome all the difficulties inherent to its peculiar surroundings.

The larva is amphipneustic and both pairs of spiracles are well developed. The prothoracic spiracle, as has already been shown (Fig. 13, p. 15), is enclosed in a deep furrow of the segment and can be evaginated only by a gradual and strong compression of the body. The long felt chamber (*f.ch.*) gives rise to a cauliflower-like structure composed of numerous spiracular papillae (*sp.*) each bearing a minute spiracular cleft and an opening of a long duct (*d.*) of the perispiracular gland (*gl.*). The postabdominal spiracles lie in a terminal depression of the last segment and are protected by two transverse folds (dorsal and ventral) of larval integument (Fig. 43). These folds contain the perivisceral fluid of the body and their chitin is thin, soft and translucent. By an increased pressure of the internal fluid the lips of the folds become turgescent and meet along the horizontal line to close tightly over the spiracles. The closing mechanism is so perfect

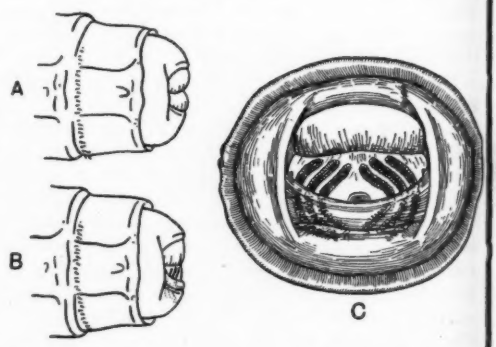


Fig. 43. Larva of *Gastrophilus*. A, side view of posterior end with lips of spiracular pouch closed; B, ibid. with lips open and showing postabdominal spiracles; C, the same as B but seen endwise (after Portier, 1911).

that no fluid or gas under pressure can break through this barrier from outside. It is somewhat surprising that the larvae completely immersed in water open the lips of the folds and expose the spiracular plate to contact with water. However, the hydrofuge secretion of the perispiracular glands protect them from penetration by water. By the play of muscles the lips of the folds may open widely, bringing the spiracular plates into contact with the surrounding medium (Fig. 43 B, C). The postabdominal spiracles are well described in papers by Enderlein and Portier. The two spiracular plates which are fused along their internal borders show a single external scar in the centre of the plate and two groups of three elongated arc-like peritremes on its left and right sides. The spiracular plate is supported internally by a thin layer of chitinous felt which is followed by a spiracular chamber, the distal end of which receives five pairs of tracheae of unequal calibre and length. Of these tracheae, one pair which

is distinctly visible in a dissected larva (Pl. I) and is of smaller diameter, represents the main tracheal trunks (*Tr.*). They communicate anteriorly with the prothoracic spiracles (*p.s.*) and supply the tracheae to all organs of the larva. The other four pairs, which are exceptionally wide at their base, rapidly decrease in diameter, thus acquiring a distinctly conical shape. All along their surface they give off numerous branches of smaller tracheae which ramify and finally break up into numerous tufts of intraprotoplasmic tracheoles filling the large tracheal cells (Fig. 44 A, B). The latter, which are of a distinctly red colour owing to the presence of oxyhaemoglobin (*t.h.c.*, Pl. I), are very numerous and mask completely the conical tracheae which can be seen only after removal of these cells.* In a much lower concentration haemoglobin is also present in cells of the fat body, the muscles and the proventriculus.

These haemoglobin containing tracheal cells enable the larva to make better use of an intermittent contact with air bubbles by taking up a much larger amount of oxygen than is necessary for their immediate requirements. The oxygen is used during intervals when the larva fails to secure contact with air bubbles.

* A more detailed account of the structure, development and function of these cells will be given separately in a paper devoted to this subject.

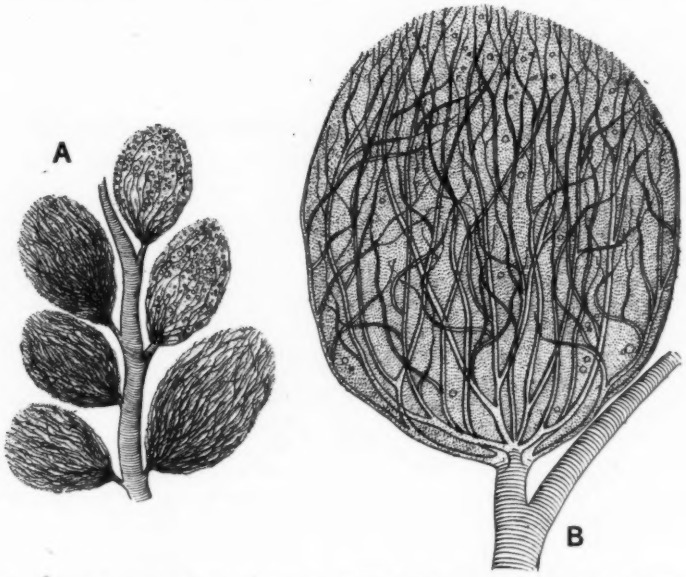


Fig. 44. *Gastrophilus intestinalis*. A, bunch of tracheal haemoglobin-containing cells. × 85. B, tracheal cell showing intracellular tracheoles. × 200.

XVI. RESPIRATORY ADAPTATIONS OF LARVAE OF VIVIPAROUS DIPTERA DURING INTRA-UTERINE LIFE

Viviparity among Diptera is not uncommon, but its evolution in different families did not follow the same path nor was it promoted by similar causes. The viviparous forms must therefore be considered as being of polyphyletic origin. Moreover, Diptera provide a remarkable series of transitions between the ordinary oviparous and the highly specialized pupiparous species. The main biological groups of this series are characterized by females (1) laying eggs developing outside their body, (2) retaining eggs until the formation of the larvae which emerge immediately after oviposition, (3) laying first stage larvae which emerge but do not grow within the uterus of the female and finally, (4) laying fully developed larvae which feed and grow within the uterus and turn into pupae soon after being laid. Only the larvae belonging to the last group will be considered in this chapter.

The proper study of the larvae of pupiparous Diptera begins only with the work of Leuckart (1858) and Pratt (1893) on *Melophagus ovinus* L. But it is mainly to Pratt that we owe the final demonstration of a number of important facts which can be summarized as follows: (1) the larvae of *Melophagus* are similar to those of other cyclorrhaphous

Diptera, (2) they pass within the uterus through three larval stages separated by two moults, (3) their midgut is in the form of a large sac filling almost completely their body cavity, (4) there is no communication between the midgut and hindgut, (5) salivary glands are absent, (6) the larvae feed upon substances secreted by the accessory glands which open into the uterus close to the buccal opening of the larva, and (7) they are metapneustic and are provided with a normally developed tracheal system.

These important observations were confirmed by Minchin (1905), Stuhlmann (1907) and especially Roubaud (1909) on the larvae of *Glossina*. According to Roubaud the larvae of *G. palpalis* emerge within the uterus where they feed and grow, undergoing, like the majority of cyclorrhaphous Diptera, two moults. The structure of their alimentary canal resembles that of *Melophagus* and they feed by sucking the papilla projecting within the uterus and bearing the openings of nutrient glands (Fig. 45). The larva is metapneustic with, the postabdominal spiracles directed towards the vagina of the female.

The study of other pupiparous forms amply confirmed the main results of the work summarized above. It is well established now that in all these forms the small larva which emerges from an ordinary egg within the uterus of the female is a typical larva of a cyclorrhaphous fly. It feeds and grows within the uterus, and although it shows marked characters of 'parasitic adaptation' as far as it concerns the general appearance and the alimentary canal, it has nevertheless a well-developed metapneustic respiratory system and it depends on the external air. The unusual development and hardening of the postabdominal spiracles in *Glossina* (Fig. 46 A, *sp.*) and certain Pupipara enable these larvae to distend the uterine and vaginal apertures of the female, thus establishing an easy contact with the air.

Although the postabdominal spiracles were known in all these larvae, their intimate structure was understood and described for the first time by Newstead (1918). He was able to show that the anal lobes of *Glossina* larva are not protective organs of spiracles as was believed by Roubaud, but are the spiracles proper of the larva. However, not being aware of a previous description by Nielsen (1909) of somewhat similar structures in other dipterous larvae (see Figs. 8 E, F, p. 10), Newstead described the spiracles of *Glossina* and of pupiparous larvae under the name of 'polypneustic lobes'. In *Glossina* larvae each lobe is covered by numerous spiracular papillae touching each other. These observations were confirmed in the course of the present investigation. The felt chamber of each spiracle was found to divide into three main branches widening at their apex and giving rise to secondary branches which ramify and terminate in small papillae covering the surface of the spiracular lobe (Fig. 46 A–C). On the other hand, the 'paired abdominal spiracles' described and figured by Newstead as small protrusions lying about the middle of the inner surface of the lobes are not spiracles but external scars of the ecdysial openings through which the tracheae of the previous stage were expelled. The larvae of hippoboscids resemble those of *Glossina* in having very large postabdominal spiracles forming one highly sclerotized structure occupying the posterior end of the body (Fig. 47). The felt chamber divides here into three main branches which give off the spiracular papillae. A similar structure of the spiracles was found in the larvae of *Lynchia maura* by Newstead and in those of *Ornithomyia strigilecula* by

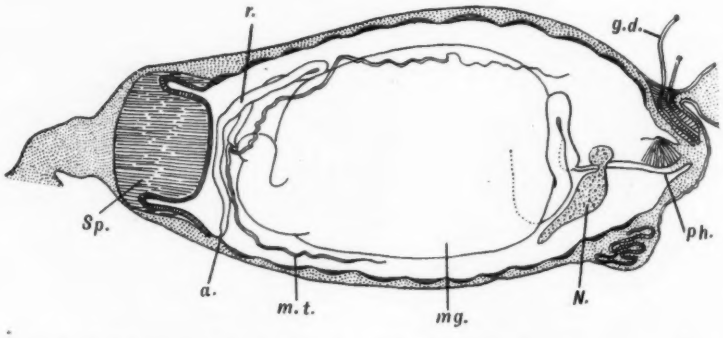


Fig. 45. *Glossina palpalis*. Larva of the tse-tse fly within its mother's uterus. *a.* anal opening of larva; *g.d.* glandular duct of fly; *m.g.* midgut of larva; *m.t.* malpighian tubes; *N.* central nerve system; *ph.* pharynx; *r.* rectum or hind-gut; *Sp.* spiracular lobes (after Roubaud, 1909). × 25.

Ferris (1923). The respiratory system in the larvae of a few other Pupipara shows certain interesting peculiarities which can be mentioned now:

(*a*) The postabdominal spiracles in *Melophagus*

scar filament (*sc.f.*) connects the basal portion of the felt chamber with the spiracular depression.

(*b*) The respiratory system of *Cyclopodia greeffi* Karsch described by Rodhain & Bequaert (1916)

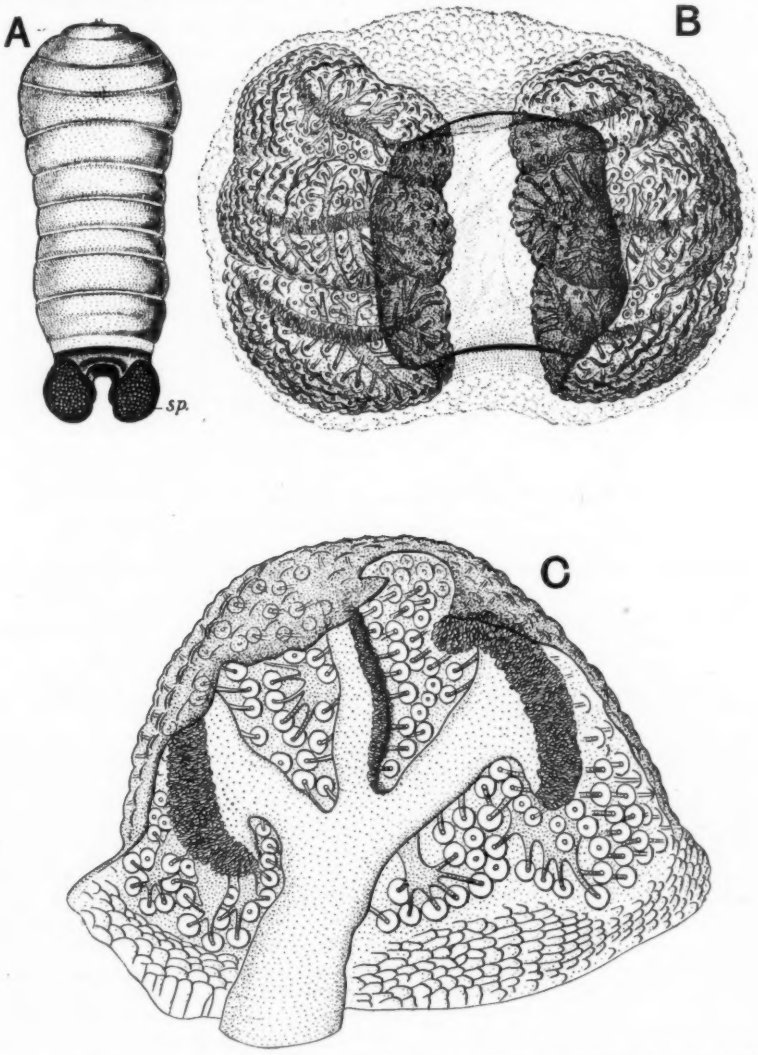


Fig. 46. *Glossina palpalis*. A, full-grown larva (after Roubaud, 1909); B, postabdominal spiracles, end view; C, dissected portion of the latter seen from inside. A × 9, B × 55, C × 85.

ovinus (Fig. 48 A) show two circular shallow depressions (*dep.*) of sclerotized chitin. The felt chamber divides into three spiracular branches (*sp.b.*) of which two terminate in spiracular papillae within the depression and one outside it. A short

shows two pairs of small spiracles (Fig. 48 B, *a.*, *p.*) lying in the posterior region of the dorsal surface of the larva. The short and bent tracheal trunks (*tr.*) form near their anterior ends a small air pocket (*a.p.*) which gives rise to a longi-

tudinal branch directed anteriorly (*l.b.*), the short tracheal trunks are connected by a transverse commissure (*cm.*) arising just behind the anterior spiracles. As the peripheral region of the larva shows clear indications that the body is composed of eleven segments, the anterior and the posterior pairs of that observed by Thorpe (1941) in the pupa of *Cryptochaetum striatum* (cf. p. 46 and Fig. 38 E).

(*c*) The pupa of *Aspidoptera megastigma* (Speiser), described by Ferris (1923) shows three large heavily sclerotized spiracles arranged in a triangle on the dorsal side of the posterior half of the

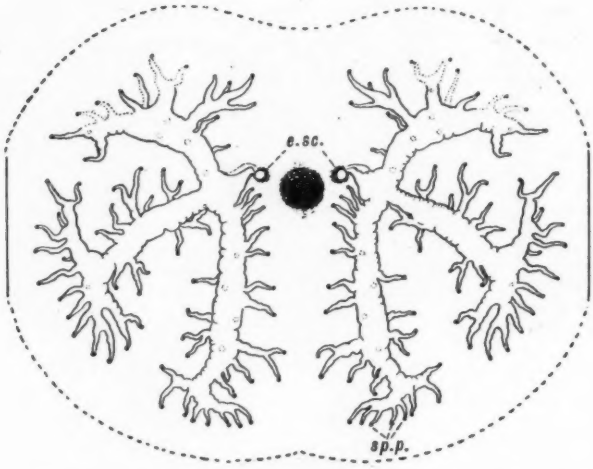


Fig. 47. *Hippobosca maculata.* Postabdominal spiracular plate of full-grown larva; *e.sc.* external scar; *sp.p.* spiracular papilla (after Newstead, 1918).

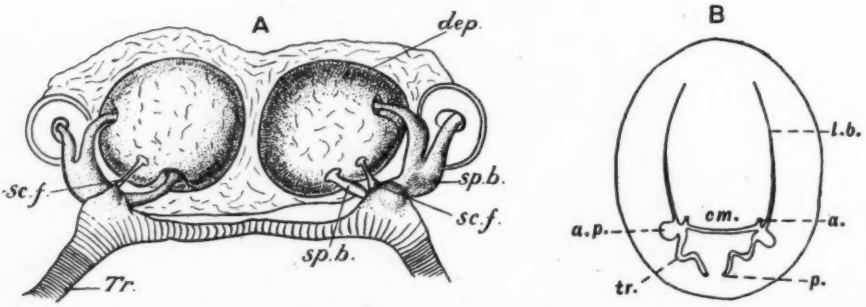


Fig. 48. A, *Melophagus ovinus*, postabdominal spiracles of full-grown larva seen from inside. *dep.* cup-like depression; *sc.f.* scar filament; *sp.b.* spiracular branch; *Tr.* tracheal trunks. B, *Cyclopodia greeffi*. Full-grown larva; *a.* anterior spiracles; *a.p.* air pocket; *cm.* transverse commissure; *l.b.* longitudinal tracheal branch; *p.* posterior spiracles; *tr.* tracheal trunks (B after Rodhain & Bequaert, 1916). A × 87, B × 10.

spiracles must belong to the prothoracic and the eight abdominal segments respectively. The proximity of the two pairs of spiracles and the posterior position of the prothoracic pair can be explained by the very unequal development of the dorsal and ventral sides of each of the segments, or to a contraction of the dorsal side somewhat similar to body (Fig. 49 A, B). The anterior pair is undoubtedly the prothoracic one (*p.sp.*), while the posterior single spiracle which is distinctly larger than the others is formed by the fusion of two postabdominal spiracles (*a.sp.*). Each spiracle (B) shows a number of small spiracular papillae arranged in a row, forming an almost complete circle. The posterior position of the

prothoracic spiracles is probably caused by an unequal development of the dorsal and ventral sides of the segments.

The study of the larvae of viviparous Diptera clearly shows that while they feed and grow within the uterus of their mother their respiration does not differ much from that of the free-living larvae of cyclorrhaphous Diptera.

its prostomium, perforates it and thrusts its spiracles through the aperture. The larvae parasitic in arthropods perforate from inside either the cuticle of the body or of the tracheae in order to bring their postabdominal spiracles in contact with air. The migration of *Hypoderma* larva is very long both in terms of distance and time. On reaching the back of the host the larva perforates the skin from inside in order

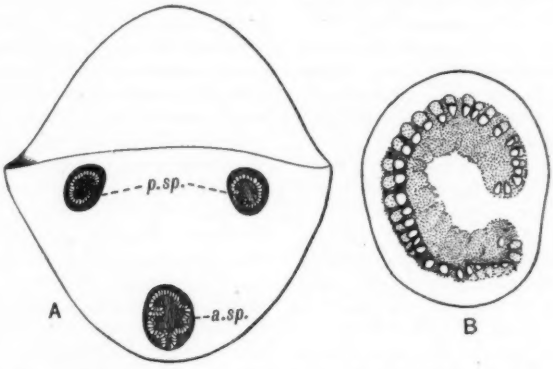


Fig. 49. *Aspidoptera megastigma*. A, pupa, dorsal view; B, prothoracic spiracle. *p.sp.* prothoracic spiracle; *a.sp.* abdominal spiracle (after Ferris, 1923).

XVII. RESPIRATORY ADAPTATIONS OF PARASITIC LARVAE OF DIPTERA COMPARED WITH THOSE OF HYMENOPTERA

It is remarkable that the parasitic larvae of Diptera have a well-developed tracheal system which is either meta- or amphipneustic. With a few exceptions the parasitic larvae pass through a more or less long period of life when they are completely enclosed within their host, occupying different positions such as the body cavity, the skin, the muscle, nerve and connective or glandular tissues. During this period they feed and grow very little or not at all, and their respiratory activity depends upon the oxygen dissolved in the surrounding medium, the gas exchange taking place across the whole surface of their body. This gas exchange, however, soon becomes insufficient for their growth and activity, and the larvae move from their initial position in order to bring their postabdominal spiracles in communication with air. In exceptional cases like that of *Melinda cognata* parasitic in snails, the postabdominal spiracles are already in contact with the air of the pulmonary cavity of the host, and when the snail retracts the larva pushes the posterior end of its body through the pneumostome of the host. In all other cases, however, the larva has to make a respiratory aperture at a suitable point in the host's body. Thus in *Pollenia*, parasitic in an earthworm, the larva migrates from the 15th segment of the host towards to bring its postabdominal spiracles in contact with air. The reaction of the host (except arthropods) to the formation of the respiratory aperture is of an inflammatory nature accompanied in the case of the earthworm by a complete necrosis of surrounding tissues. The reaction of an arthropod host to the formation of such an aperture consists in the proliferation of hypodermal layer and the secretion of a thin chitinous sac enclosing almost completely the parasitic larva; this sac is more strongly sclerotized near the aperture forming a real chitinous siphon within which is engaged the last abdominal segment with its pair of functional spiracles.

The respiratory adaptations of parasitic larvae, apart from these more or less general aspects, may exhibit a few more specific characters proper only to a few cases. To these belong, for instance, (1) the long postabdominal appendages acting as tracheal gills in the larvae of *Cryptochaetum*, (2) the haemoglobin-containing tracheal cells of *Gastrophilus*, (3) the postabdominal spiracular pouch of *Gastrophilus*, (4) the postabdominal spiracular siphon of *Ginglymyia* (cf. p. 47) and others. The dominating factor in the respiratory adaptation of parasitic dipterous larvae is that usually they cannot feed and grow actively without bringing their highly developed tracheo-spiracular system into communication with air. This is reflected in certain structural features of these larvae, their behaviour within the host and their life histories.

In this respect a comparison of parasitic adaptation in Diptera with that in Hymenoptera is very instructive. As the study of Hymenoptera lies outside the scope of this paper only the main features of their parasitism can be briefly enumerated here:

(1) The range of hosts of parasitic Hymenoptera is much narrower than that of parasitic Diptera, being restricted among animals only to arthropods.

(2) Their respiratory system, at least in full-grown larvae, is generally of a polypneustic type, and the tracheal trunks are not well developed (except in *Chalcis fonscolombei* Duf. (Parker, 1924)).

(3) When the respiratory system is oligopneustic it does not show a marked compensatory development of the functional spiracles.

(4) The larva never produces a respiratory aperture either in the integument of the body or the tracheae of its host in order to bring its spiracles in contact with air.

(5) When the contact between the spiracles and the outside air is established, its mechanism is very different from that found in Diptera. In fact, it is with the hollow stalk of an egg left protruding outside the body of the host that the larvae of certain chalcids bring their postabdominal spiracles in contact with the air (Imms, 1918; Parker, 1924; Thorpe, 1936). In only one chalcid, *Encyrtus inflexus* parasitic in a coccid (*Saissetia hemispherica*), a more intimate connexion was described between the spiracles of the parasitic larva and the tracheae of the sheath (of phagocytic origin) surrounding it (Thorpe, 1936).

(6) No chitinous sac of hypodermal origin was found to surround the parasitic larvae of Hymenoptera.

(7) The hymenopterous larvae, not being very active, have comparatively low respiratory requirements which are easily satisfied by the amount of oxygen dissolved in the surrounding medium.

(8) The gas exchange is greatly favoured by the thin cuticle of the body and the few accessory structures such as a highly developed net of peripheral tracheae in the first stage larvae of *Macrocentrus gifuensis* Ashm. (Parker, 1931) or the bladder-like protrusion of postabdominal segment in *Apanteles* and *Microgaster* (Thorpe, 1932).

On the whole the respiratory requirements of parasitic hymenopterous larvae, unlike those of dipterous larvae, affect very little their interrelationship with the host or their life history.

XVIII. RESPIRATORY ADAPTATIONS OF DIPTEROUS PUPAE

(*a*) *Prothoracic spiracles of orthorrhaphous pupae*

In dealing with this problem, the following few points must be kept in mind:

(i) The thoracic portion of the pupa is marked by its bulk and its structural differentiation.

(ii) The orientation of the pupa within its medium must be such as to favour the emergence of the adult fly which is a purely terrestrial organism.

(iii) This orientation is facilitated by the fact that the pupa takes no food so that its respiratory requirements are completely independent of a nutrient medium. It is therefore not surprising that the orientation of the pupa, especially in a fluid medium, is usually the reverse of that of the larva, and that the predominant spiracles in the pupa are the prothoracic and not the postabdominal pair, as is usual in the case of the larva.

The study of prothoracic spiracles in pupae begins with important observations by Réaumur (1738) which were followed by numerous descriptions scattered in the entomological literature and a comprehensive study of the subject by de Meijere (1902) who gave the first correct description of this organ and of its main structural variations. Many interesting examples of prothoracic spiracles in pupae can be found described and figured in several papers dealing with the structure of larvae and pupae of Diptera, but more specially in papers by de Meijere (1916), Malloch (1917), Alexander (1920) and Johannsen (1934, 1935, 1937 and 1937*a*). A mere glance at the figures in these few papers gives a clear idea of the great variations in the size, shape and structure of the prothoracic spiracles described as prothoracic trumpets, horns, siphons or gills. They can either be very short, sessile and hardly perceptible or distinctly prominent and in some pupae (*Ptychoptera*) reaching a very great length. In these cases only one of the spiracles grows into a siphon longer than the rest of the body while the second spiracle is more or less atrophied.

In other pupae the prothoracic spiracles are in the form of branched tracheal gills (Simulidae) or tufts of numerous filaments (certain chironomids). We shall examine here only the respiratory adaptations of the pupae of *Taeniorhynchus* and of certain cyclorrhaphous Diptera. The pupa of *Taeniorhynchus* (Edwards, 1919; Wesenburg-Lund, 1918) like its larva is adapted to a completely submerged mode of life. The prothoracic spiracles are in the form of horns drawn out into sharp points which are inserted into the tissue of aquatic plants from which the pupa obtains the necessary supply of oxygen (Fig. 50 A, B). The prothoracic spiracles of the pupa act therefore in the same way as the postabdominal spiracles of the larva (cf. pp. 38–9). The insertion of the prothoracic spiracles into the plant roots, which was observed by Galliard (1934), takes place immediately after the splitting of the larval skin and the liberation of the anterior portion of the pupa, while the rest of its body remains still within the larval skin which, by means of its postabdominal siphon, is anchored

on to the plant. This gives the pupa the necessary support while it thrusts the prothoracic spiracles into the plant tissue. As to the mode of emergence of adult mosquitoes, Edwards (1919, p. 87) writes as follows: '...several specimens of *T. richiardii* hatched out in a pail which I had brought home with pond-water and sods of grass. The empty pupal skins were found floating on the surface of the water, and the pupae must therefore have risen to the surface for the emergence of the adult. An examination of these skins showed that in every case the terminal portions of the breathing-tubes were missing, hence it must be inferred that they had been left behind in the roots. Most likely their barbed structure, while facilitating their insertion, would prevent their being withdrawn. There seems to be a line of weakness at the base of the barbed portion which might render its detachment easy.'

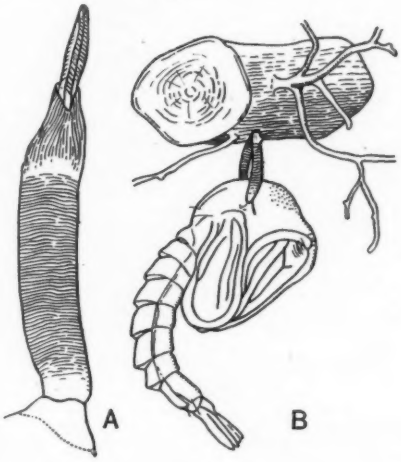


Fig. 50. *Taeniorhynchus richiardii*. A, prothoracic horn of pupa; B, pupa attached to roots of submerged plant (after Marshall, 1938).

(*b*) *Prothoracic spiracles of cyclorrhaphous pupae*

The pupae of cyclorrhaphous Diptera are always enclosed within the cuticle of the larva, sclerotized into a hard and dark case known as the puparium.

The formation of the puparium is always accompanied by a more or less complete evagination of larval prothoracic spiracles, especially if they are lying within a deep furrow of the first thoracic segment. This can be seen clearly in the puparium of *Drosophila*, *Gastrophilus*, *Dermatobia* (Fig. 42, p. 50) and especially in that of *Aulacogaster rufitarsis* (Fig. 51 A, B) which are found in the exudates from the wounds of trees.

The pupa is, however, often smaller than the puparium, and the space between the two cuticles, especially in the anterior region, is filled with air. In the majority of acalypterates the pupa has a pair of prothoracic spiracles which are enclosed within the puparium and are known as internal spiracles. Each spiracle is composed of a more or less protruding felt chamber covered with numerous spiracular papillae. In the great majority of calypterates the felt chamber of the prothoracic spiracle of the pupa, in addition to the internal spiracle, is drawn out into a more or less developed and sclerotized horn covered with spiracular papillae and protruding outside through a small circular aperture in the wall of the puparium.

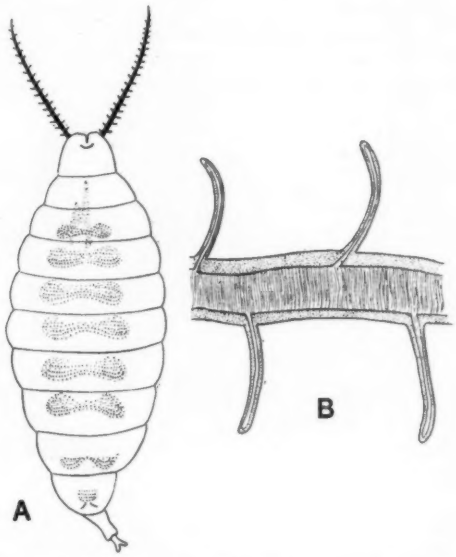


Fig. 51. *Aulacogaster rufitarsis*. A, puparium with evaginated prothoracic spiracles of larva; B, detailed structure of these spiracles. A × 24, B × 240.

The structure of such a prothoracic spiracle was well described by de Meijere (1902) and can be easily seen in the pupae of certain anthomyids (Keilin, 1917) (Fig. 52) such as *Graphomyia maculata* Scop. (A, B) or *Myospila meditabunda* F. (C). The most remarkable phenomenon in the respiratory adaptation of the pupa is the protrusion of the prothoracic horns through the hardened cuticle of the puparium. This is made possible by the presence in the wall of the puparium of two small circular spots where the cuticle escapes hardening, remaining thin, clear and very fragile. These spots were first noticed by Réaumur (1738) in the puparia of *Eristalis* as two small circles more white than the rest and surrounded by a brown ring. On opening the puparium

he found that the horns lie parallel to the length of the body while after protrusion they become perpendicular. Dufour (1840) described them in pupae of *Phora*, *Aricia* and other Diptera. Laboulbène (1861) was greatly impressed by the discovery upon the puparium of *Tachina villica* Rob. Desv. of the minute prothoracic respiratory horns of the pupa.*

In 1870 Perris found the prothoracic horns in pupae of *Xylota pigra* Meig. and *Aphiochaeta pusilla* Meig. and described for the first time their extrusion through the walls of the puparium.†

The best material for the study of the prothoracic horns and their extrusion is presented by phorids such as *Paraspiniphora bergenstammi* Mik. or *P. maculata* Meig.

On breaking a shell of a dead garden snail, *Helix aspersa*, which harbours the larvae of these phorids, one can often see a large number of small slightly curved rods arising from a thick brown sticky substance covering the internal surface of the shell. After washing off the sticky remains of the dead snail it becomes apparent that the rods are the prothoracic horns originating in pairs from the dorsal side of the pupae, which by the ventral surface of their abdominal segments are firmly attached to the shell (Fig. 53). This attachment takes place just before pupation, when the larva retracts the anterior and posterior segments of the body and its cuticle becomes gradually sclerotized, turning from white to yellow and brown, leaving only two spots of clear thin chitin. About 24 hr. after pupation the anterior end of the pupa is contracted leaving a free space within the anterior chamber (*a.ch.* Fig. 54 A and Pl. II A). On the right and left sides of the pupa appear the prothoracic horns (*h.p.*) which emerge from apertures of their imaginal disks which are not yet completely evaginated. These horns are

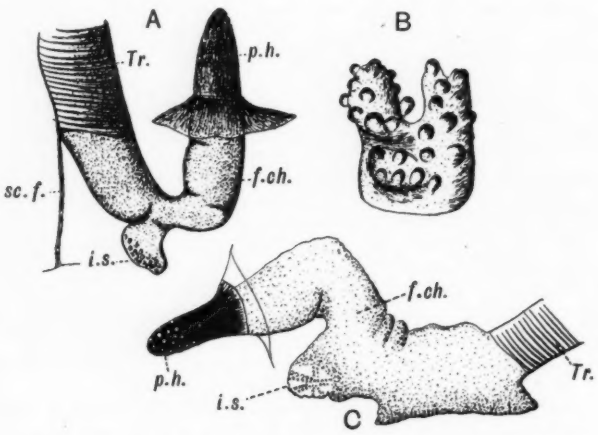


Fig. 52. Prothoracic spiracles of pupae of Anthomyidae. A, *Graphomyia maculata*; B, internal spiracles of the pupa; C, *Myospila meditabunda*. *f.ch.* felt chamber; *i.s.* internal spiracles; *p.h.* prothoracic horns; *sc.f.* scar filament; *Tr.* tracheal trunks (after D. Keilin, 1917). A ×90, B ×525, C ×150.

* Having grasped the physiological significance of this organ Laboulbène tells the reader how thrilled he was in making this observation. He writes: 'C'est pour moi un vrai bonheur de trouver sur la pupe de la *Tachina villica*, ce vestige d'un organe arrivé au summum de développement pour les Muscides chez les pupes des *Phora*. Il faut avoir tourné et retourné dans la main ces berceaux d'une simple Mouche, pour comprendre la joie qu'éprouve l'observateur qui finit par découvrir sur ce corps inerte la trace d'un organe dont l'importance physiologique est si haute. Cette petite saillie, ce point élevé, si insignifiant pour le vulgaire, nous révèle le mode de formation des cornes dorsales des pupes chez les *Aricia*, les *Eristalis*, les *Eumerus*, le *Phora*, etc.'

† His description of this phenomenon reads as follows: 'Près du bord antérieur du 4e segment, aux endroits où les stigmates se font jour habituellement, j'ai aperçu deux petites places plus claires que le reste de l'enveloppe, ce qui me fait supposer qu'en vue de favoriser la sortie des stigmates, la nature toujours consequente dans ses desseins, a donné sur ces points une organisation spéciale à la peau larvaire. A travers l'enveloppe j'ai vu la nymphe déjà formée, et implantées sur le dos du thorax, deux pièces, noires, dirigées vers la tête, qui n'étaient autre chose que les deux stigmates faisant effort pour sortir. De temps en temps de petits mouvements se manifestaient dans l'intérieur de la pupe. Enfin, à la suit d'un de ces mouvements, les pointes de deux stigmates ont coincidé avec les deux endroits plus clairs dont j'ai parlé et en quelques secondes ces stigmates étaient en dehors.'

still of a light brown colour and show distinctly the small spiracular papillae (Fig. 54 B, *sp.*). The prothoracic horns lie directly under the dorsal wall of the puparium, being parallel to its surface and pointing anteriorly. Their ends move gradually forward within the anterior chamber of the puparium owing to their growth and evagination of disks from which they originate. About 45 hr. from the onset of pupation the horns assume their final shape and structure reaching with their tips the anterior end of the puparium (Pl. II A). Ten hours later the body of the pupa suddenly contracts dragging the pro-

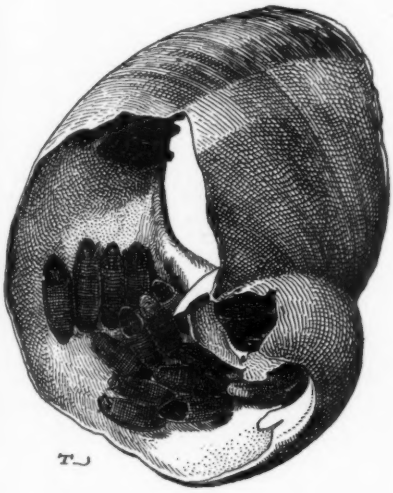


Fig. 53.

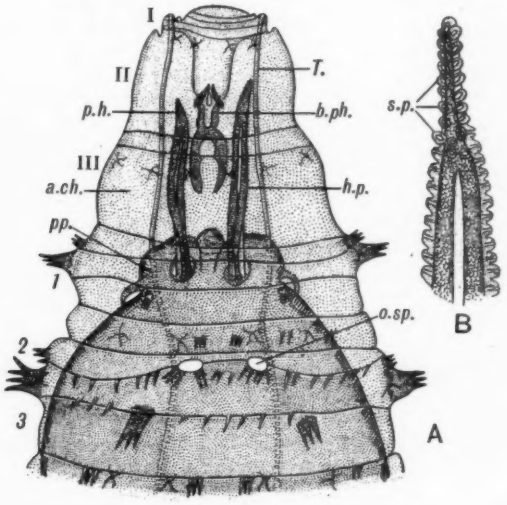


Fig. 54.

Fig. 53. Shell of *Helix aspersa* with upper whorl broken, showing pupae of *Paraspiniphora maculata* Meig. fixed to the inner side of shell. × 2.

Fig. 54. *Paraspiniphora bergenstammi* Mik. A, anterior portion of a young puparium; B, detailed structure of prothoracic horn. *a.ch.* anterior chamber of puparium; *b.ph.* bucco-pharyngeal armature of larva; *h.p.* prothoracic horns of pupa before protrusion; *o.sp.* fragile spots on chitin of puparium through which are ultimately thrust the prothoracic horns; *p.h.* = *h.p.*; *p.p.* pupa contracted within puparium; *s.p.* spiracular papillae; I, II and III, three thoracic segments; 1, 2, 3, abdominal segments of puparium. A × 37, B × 200.

thoracic horns backwards until their extremities appear just behind the two clear spots (*o.sp.*) of the puparium (Pl. II B). The pupa now makes a few jerking movements forward and backward, striking with the tips of the horns the thin chitinous membranes of the clear spots until they are perforated. A few more movements of the pupa and the horns are thrust through the apertures thus made, to be completely extruded from the puparium (Pl. II C). The whole of this procedure is completed within 1 min. That the contraction of the pupa is due mainly to the evagination of the imaginal disks of the head, wings and legs was suggested already by Réaumur (1738) in his important study of *Eristalis*.*

It is perhaps one of the most remarkable phenomena observed in insects that the contraction of the pupa, due to evagination of the imaginal disks, the length of the prothoracic horns and the predetermined clear fragile spots on the 1st abdominal segment of the puparium are all adjusted in such a way that the tips of the horns having moved about 1500 μ backwards are brought directly against the two minute fragile windows, through which they must break. In spite of this delicate adjustment, the extrusion of prothoracic horns is almost always successful. However, occasionally one or both horns fail to perforate the clear spots and slip forward

* In dealing with this subject Réaumur (1738, vol. IV, p. 465) writes: 'Si on se rappelle qu'il y a au bout antérieur de l'insecte une petite cavité de dedans laquelle sortent successivement les jambes, les ailes et la tête de la mouche, on concevra aisément que lorsque de pareilles parties ont commencé à sortir du bout antérieur de notre dernier insecte, ses cornes ont dû être poussées en arrière et redressées; les parties molles et comme charnues qui de l'intérieur de l'insecte son venues en dehors, ont dû produire tout ce jeu.'

remaining enclosed within the puparium. If one prothoracic horn is extruded the pupae may develop normally, if both horns fail to be extruded the pupa does not develop.

XIX. ORIGIN OF DIFFERENT RESPIRATORY SYSTEMS

(*a*) *Holopneustic systems*

It is conceivable that the primitive form of a respiratory system in insects was composed of ten pairs of very similar tracheo-spiracular metameres originating in ten pairs of spiracles equal in size and similar in structure lying on intersegmental membranes. This primitive holopneustic system can be described as isopneustic, and the first modification in its structure probably consisted in the gradual migration of spiracles from intersegmental regions upon the segments.

This migration in dipterous larvae has taken place in two opposite directions: the first pair has moved forwards while the other pairs moved backwards, thus leaving the mesothoracic segment free of spiracles.

The changes in the external conditions of life of these larvae and their gradual adaptation to a more or less fluid medium is the principal cause of all the subsequent specialization of their respiratory systems. In fact, it becomes difficult for those larvae which seek their sustenance in a compact, viscous or semi-fluid media to have all their spiracles equally well exposed to air. It is always one extremity of the body or the other that the larva can most readily protrude from the medium where it lives, in order to maintain communication with the open air. The terminal spiracles are, therefore, more often functional than those of the intermediate segments. This greater activity probably influences the metabolism of the hypodermic layer of terminal spiracles which, prior to each moult proliferates more rapidly than does the hypoderm of the intermediate spiracles. In this manner the terminal spiracles become gradually larger and more complicated in structure; thus the respiratory system becomes holopneustic heteropneustic as shown by the larvae of Bibionidae.

(*b*) *Peripneustic system*

The next step in specialization consists in the closing of the metathoracic spiracles and the appearance of the peripneustic respiratory system so widely distributed among insects. It is, however, difficult to explain the obliteration of spiracles in a segment lying close to other segments which are provided with functional spiracles. So far the only plausible explanation of this phenomenon was suggested by Weber (1927) and based upon the study of larvae and pupae of Lepidoptera. In the obtect pupa of these insects the second or metathoracic pair of spiracles is completely covered by wings tightly applied and cemented down to the body. These spiracles having lost the communication with the air, become less functional and their development becomes more and more delayed. This delay gradually extends to the larval stages with the result that metathoracic spiracles remain obliterated in all larval stages giving rise to the peripneustic system.

This interesting explanation remains, however, to be tested by a careful comparative study of holopneustic and peripneustic larvae and pupae belonging to other orders of insects.

(*c*) *Hemipneustic system*

The hemipneustic derives from the peripneustic system through a loss of the postabdominal spiracles. This view is strongly supported by the appearance of the metapneustic stage during the first stage of development in both peripneustic and hemipneustic systems. The origin of the hemipneustic system in mycetophilid larvae can be explained by their mode of life. In fact, the great majority of these larvae are fungivorous and are found within the spongy tissues of fungi which are permeated with air spaces. Under such conditions the anterior segments of the larva are in a more favourable position to establish contact with air spaces; the remaining portion of the body, and especially the posterior end, lies in the burrow, the walls of which usually undergo rapid liquefaction. This probably accounts for the greater development of the prothoracic and the closing of the postabdominal spiracles in all the hemipneustic mycetophilid larvae.

(*d*) *Oligopneustic systems* (*amphipneustic, metapneustic and propneustic*)

The study of the development of tracheo-spiracular systems showed that the spiracles which are more prominent and functional in the full-grown larva are the first to appear during the successive larval stages, while the other spiracles follow them after a delay of one, two or even three moults. Thus in a peripneustic larva of *Scatopse notata* the seven pairs of abdominal spiracles become functional only in the third stage, while the same spiracles in a hemipneustic mycetophilid larva open in the fourth stage larva only.

If owing to a more fluid medium the seven abdominal segments remain more frequently submerged and the delay of development of their spiracles extends over two more larval stages, a full-grown larva with an amphipneustic respiratory system will be obtained. The seven pairs of abdominal spiracles will proliferate and become functional in the pupa and in the adult insect. A delay in the development of seven abdominal

pairs of spiracles by only one larval stage in the hemipneustic system will give rise to a full-grown larva with a propneustic respiratory system.

In the same manner can be explained the development of the metapneustic from the amphipneustic system.

That the oligopneustic respiratory systems may arise from the polypneustic system owing to a gradual delay in proliferation of certain spiracles is supported by the following consideration. If, in a group of larvae, the respiratory system of which belong to one general type, we find exceptional larval forms with another type of respiratory system, the latter will be similar to one of the respiratory systems which is met with during the developmental stages of the general type. This can be illustrated by the following few examples:

(1) Mycetophilid larvae are usually hemipneustic except the larvae of *Diadocidia* and *Speolepta* which are propneustic and *Ceroplatus* which is apneustic. We have seen, on the other hand, that propneustic and apneustic systems characterize the third and second stage larvae of hemipneustic mycetophilids.

(2) The larvae of all Psychodidae are amphipneustic except *Pericoma californica*, which according to Kellog (1901) is metapneustic. Among the larvae of cyclorrhaphous Diptera, which are usually amphipneustic, several forms are known (cf. p. 29) with metapneustic respiratory system. It has been shown, on the other hand, that the metapneustic stage always appears as the first stage in the development of amphipneustic larvae of Psychodidae and larvae of cyclorrhaphous Diptera.

Finally, accidental delays in the development of a spiracle by one larval stage were observed as cases of abnormality in the larvae of *Scatopse notata* (Fig. 22 F, p. 28).

(e) *Apneustic system*

The apneustic system has probably originated independently from different respiratory systems. In the larvae of Blepharoceridae and Simulidae the apneustic system derived from a polypneustic system (peri- or hemipneustic) as in both these families the larvae are found attached to solid surfaces submerged in highly and uniformly aerated water. The facts that the larvae of Simulidae are fixed in an upright position and that their prothoracic non-functional spiracles are larger than those of the other segments suggest that these apneustic larvae probably originate from hemipneustic and propneustic forms.

That the apneustic system of *Ceroplatus* larvae originated from the hemipneustic system is clearly shown by the fact that these larvae belong to only a few species, within a large family of mycetophilids, the larvae of which are hemipneustic.

On the other hand, the apneustic system of *Chaoborus* (=*Corethra*) larvae originated from the metapneustic system characteristic of other Chaoborinae and other Culicidae. Finally, the apneustic system of the larvae of Chironomidae was considered at one time to derive from the metapneustic system because of some resemblance between these larvae and those of Thaumaleidae (=Orphnephilidae) which are metapneustic (Thienemann, 1910). However, this view was not supported by Saunders (1923) who found that the resemblance between the larvae of *Orphnephila* and those of Chironomidae is a superficial one and that there are striking differences in their main characters. It cannot therefore be excluded that the apneustic system of chironomid larvae also originates from a polypneustic system.

XX. IRREVERSIBILITY OF EVOLUTION OF RESPIRATORY SYSTEMS IN DIPTEROUS LARVAE

It is now generally accepted that the polypneustic or more primitive respiratory systems characterize the terrestrial mode of life, while the oligo- or apneustic systems which derive from it mark a subsequent adaptation of these insects to a submerged life in a fluid or semifluid medium.

This rule is, however, not without a few striking exceptions which deserve special consideration, inasmuch as they throw light on certain aspects of evolution of the tracheo-spiracular systems in larvae.

(*a*) *Apneustic terrestrial larvae of* Forcipomyia. The genus *Forcipomyia* belongs to Ceratopogonidae, a family closely allied to and at one time incorporated as a subfamily of Chironomidae. Although the larvae of these two families are usually aquatic, those of *Forcipomyia* are terrestrial and occur in dark enclosed spaces saturated with moisture and invaded with moulds which serve them as food. They are found under the loose bark of dead trees or logs and in decomposed vegetable matter. All along its dorsal side the larva of *Forcipomyia* shows a double row of lanceolate hairs bearing minute droplets of water of condensation which keep the surface of the larva always moist (Saunders, 1924). The tracheal system of the larva is well developed and very rich in peripheral tracheoles forming numerous radiating plexuses. There is no doubt that *Forcipomyia* derives from ceratopogonids with aquatic larvae and that the terrestrial habit of its larvae is a secondary readaptation to this more primitive mode of life. The larva does not revert however to a polypneustic respiratory system, it remains apneustic and adapts itself to the old conditions of life by new means forged during the periods of its more recent specialization.

(*b*) *Terrestrial metapneustic and amphipneustic wood-boring larvae*. The larvae of several tipulids and of *Trichomyia* among psychodids are found to live in galleries they bore in dead and often dry wood. These larvae remain, however, meta- and amphipneustic respectively. In spite of their terrestrial habit they show clear marks of previous adaptations to a semiaquatic mode of life which characterizes the great majority of the larvae belonging to these two families.

(*c*) *Terrestrial larvae of Syrphidae*. Among syrphids the larvae of certain eristalines like *Eristalis*, *Helophilus*, *Mallota*, etc., being completely submerged in water are provided with a long respiratory siphon bearing the postabdominal spiracles; the larvae of *Ceria* and *Xylota* which live in semifluid exudate of the wounds of trees have their postabdominal spiracles supported by a much shorter siphon. In the larvae of *Merodon* which feed upon bulbs of narcissi, turning them into a brown semifluid decomposed mass, the siphon appears as a short tubercle bearing the postabdominal spiracles. The myrmecophile larvae of *Microdon* and the aphidivorous syrphids' larvae are purely terrestrial organisms. Although they never come in contact with water, living either in dry nests of ants or upon terrestrial plants, their respiratory system is amphipneustic and their postabdominal spiracles are fused into one tubercle having the appearance of a short siphon. Both the terrestrial and the completely aquatic larvae of Syrphidae originated from forms adapted to semiaquatic life. The readaptation to the terrestrial life did not imply, however, in this as in other groups of larvae, the return to the more primitive respiratory system of terrestrial insects.

All this strongly supports Dollo's (1893, 1910) generalization that evolution is irreversible.

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EXPLANATION OF PLATES

Plate I

Gastrophilus intestinalis De Geer. Full grown larva dissected from the ventral side (with fat body and the mass of tracheal cells unfolded) showing internal organs, × 6·5. *a.m.* anterior part of malpighian tubes; *c.g.* cerebral ganglia; *f.b.* fat body—the pale pink colour of the fat body, due to haemoglobin, is omitted; *h.g.* hind gut; *i.t.* intestinal tracheae; *m.g.* mid gut forming several loops (not all loops are represented in this figure); *oe.* oesophagus; *p.m.* posterior pair of malpighian tubes; *p.s.* prothoracic spiracles; *pv.* proventriculus, showing haemoglobin; *s.g.* salivary glands; *t.b.* tracheal body; *t.h.c.* tracheal haemoglobin cells; *Tr.* tracheal trunks; *v.n.* ventral nerve mass (=condensed ventral chain).

Plate II

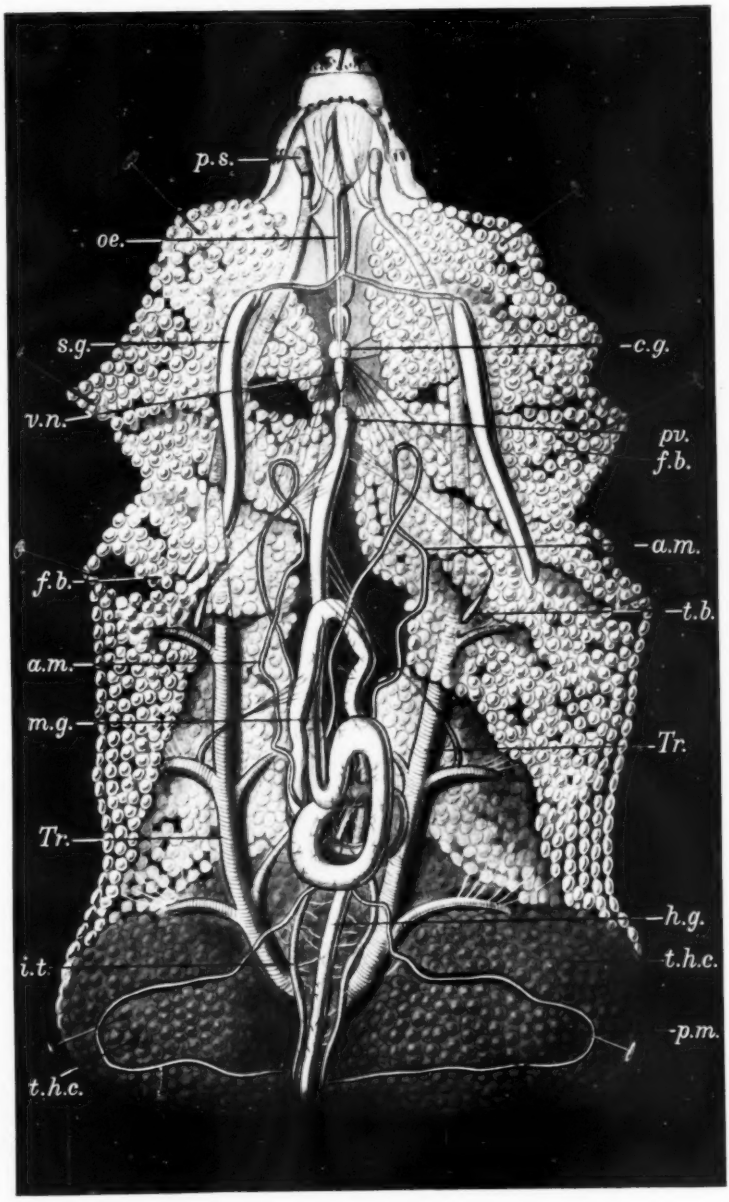
Paraspiniphora bergenstammi (Mik). Young puparia showing three successive stages in the protrusion of the prothoracic respiratory horns of the pupa, × 24.

A. Young puparium seen from the dorsal side showing by transparency the pupa and two completely formed prothoracic horns.

B. Puparium, about 52 hours old, seen from the dorsal side, showing a sudden contraction of the pupa which brings the tips of its prothoracic horns against the two clear spots of thin chitin in the wall of the puparium. The preparation represented in this figure was obtained by a rapid fixation of the contracted pupa with boiling water.

C. Puparium seen partly sideways immediately after protrusion of prothoracic horns.

a.ch. anterior chamber of puparium; *b.ph.* buccopharyngeal armature of the larva; *h.p.* prothoracic respiratory horns of the pupa; *o.s.p.* fragile spots in chitin of puparium through which are ultimately thrust the prothoracic spiracular horns of the pupa; *pp.* pupa formed within the puparium; *sp.l.* spiracles of the larva seen in the contracted posterior end of the puparium; *Tr.* tracheal trunks of the larva.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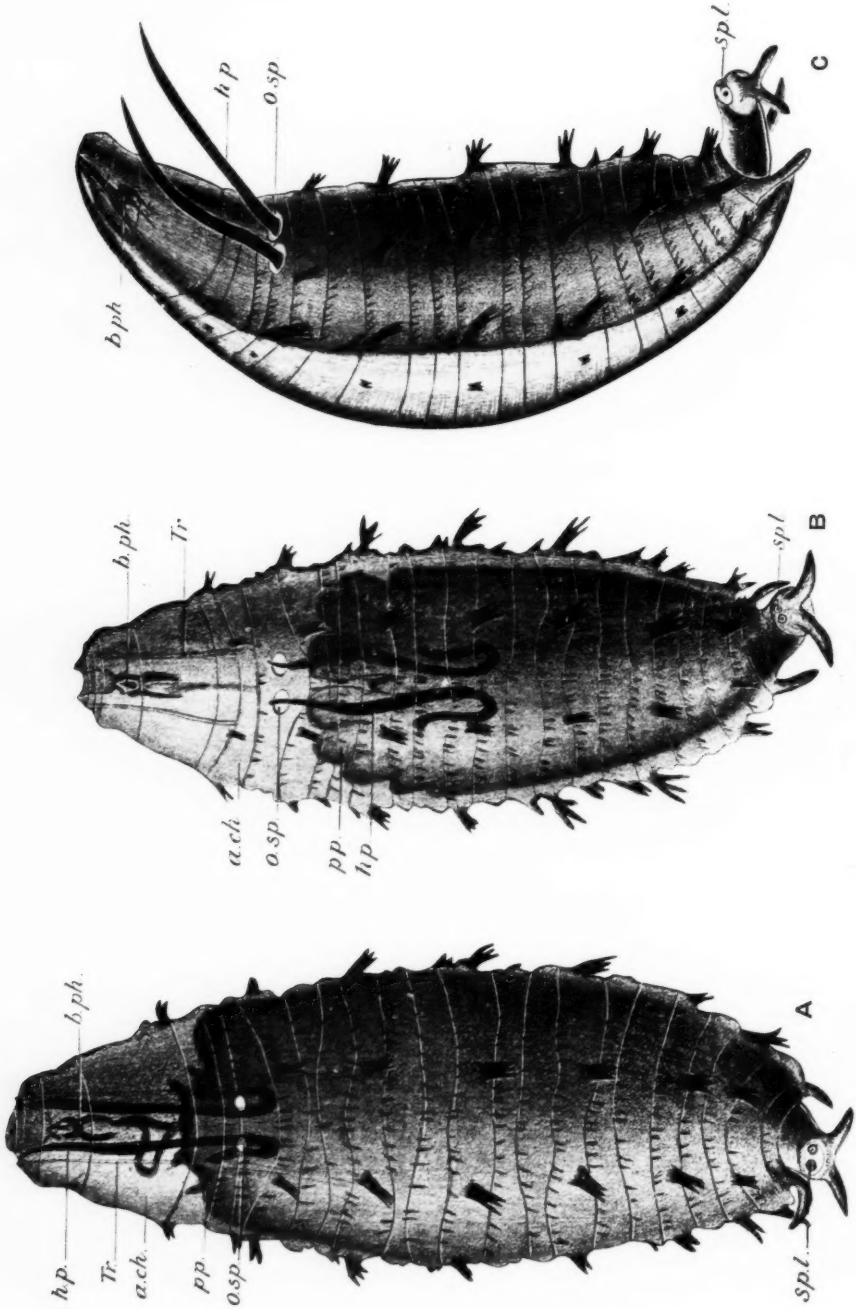
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OBSERVATIONS ON THE *SARCOPTES* OF MAN

By H. F. LUNN, B.Sc., M.B., B.S.

(With 2 Figures in the Text)

A full description of the *Sarcoptes* of the horse has been given by Buxton (1921*a*). In his subsequent remarks on the *Sarcoptes* of man, Buxton (1921*b*) notes a wide range of variation in individuals of the two varieties, but does not find any constant systematic differences between them. As a result of the study of several hundred adult female mites removed from military patients, I believe that a detailed description of the adult female *Sarcoptes* of man is unnecessary. I have found only one point of difference between my specimens and those described by Buxton. In all other respects his exact and detailed description of *S. scabiei* var. *equi* (Gerlach) applies also to *S. scabiei* de Geer var. *hominis* (Hering). Lack of experience prevents me from expressing a similar opinion regarding the male and immature stages.

MEASUREMENTS OF THE ADULT FEMALE

Forty-two specimens were measured alive. After removal from a patient, each was placed on a dry microscope slide, identified by observation of the genital apparatus, and measured at room temperature so that movement was reduced to a minimum. Results were as follows (42 specimens): length from tip of chelicera to end of body varied between 338 and 400 μ, mean length being 368 μ; S.D. 19·3. Breadth varied between 213 and 288 μ, the mean being 257 μ; S.D. 17·2. Ratio of length to breadth varied between 100/78 and 100/61, the mean being 100/70.

These figures are of the same order as those given by Buxton (1921*a*) for *Sarcoptes* of the horse, and as those quoted by Munro (1919) and Warburton (1920) for *Sarcoptes* of man. In order to demonstrate the apparent increase in size caused by the mounting of specimens, three adult females were measured first alive, then after mounting in gum Arabic medium, and finally after compression of the coverslip to exclude excess mountant. Average measurements were as follows: alive, $373 \times 270\,\mu$; after mounting, $415 \times 319\,\mu$; after compression, $457 \times 342\,\mu$. This apparent increase in size may be responsible for the wide variation in results obtained by other observers.

THE GENITAL OPERCULUM

In his description of the ventral surface of the adult female, Buxton (1921*a*) refers to a 'wide transverse flap, very shallow from before backwards', which he terms the genital operculum. He describes this operculum as covering 'the actual orifice of the tocostome...a longitudinal slit between two closely opposed...lips'.

Munro (1919) and Warburton (1920), on the other hand, apply the term tocostome to a transverse slit, immediately behind the epimeres of the anterior legs. I have been fortunate in obtaining a preparation of an adult female in the process of oviposition. The aperture through which the egg is emerging is definitely transverse rather than longitudinal (Fig. 1). In all living specimens examined

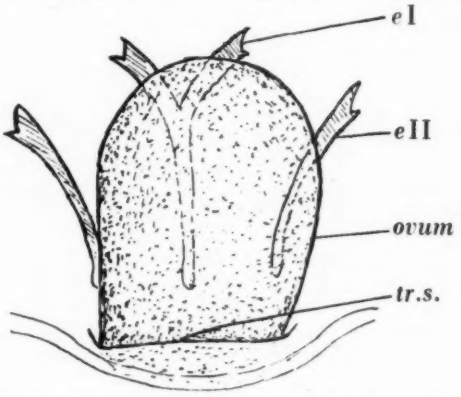

Fig. 1. Ventral aspect of adult female, drawn from specimen referred to in text. *e* I–II, epimeres of legs; V_1, first ventral seta; *t.s.* tocostomal seta; *d.a.* depressed area; *tr.s.* transverse slit (tocostome of Munro); *tr.f.* transverse fold; *l.s.* longitudinal slit (tocostome of Buxton); *ovum*, emerging egg.

since, the integument described by Buxton as the 'operculum' has been found to be depressed instead of superficial to the surrounding ventral surface (Fig. 2). This depressed area deepens backwards, and from it arise the tocostomal setae. Its posterior border is overlapped by a rounded transverse fold of integument which forms the posterior lip of the birth opening, and which has the sinuous outline described by Buxton as the free margin of the 'operculum'.

I am therefore unable to confirm that a genital operculum is present in *Sarcoptes* of man. The term tocostome seems to be indicated for the transverse

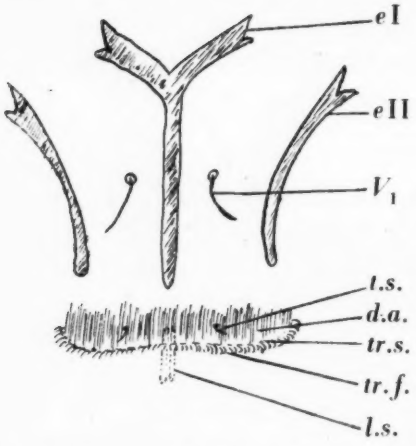

Fig. 2. Ventral aspect of adult female, semi-diagrammatic. Lettering as for Fig. 1.

slit directed downwards and forwards, which forms the external birth opening. The longitudinal slit noted by Buxton lies deep within the integument, and, in mounted specimens, bears a variable relation to the transverse slit, depending on the pressure of the coverslip. Because of its free movement, it would seem to be an internal structure, possibly a sphincter through which the egg passes before entering the birth passage.

SUMMARY

1. A study has been made of the adult female *Sarcoptes scabiei* de Geer var. *hominis* (Hering). The findings have been compared to the description of *Sarcoptes* of the horse given by Buxton.

2. Forty-two living adult female *S. scabiei* de Geer var. *hominis* (Hering) have been measured.

3. The apparent increase in size due to mounting is demonstrated.

4. The anatomy of the birth passage is discussed with special reference to the absence, in the specimens examined, of a genital operculum.

I wish to thank my A.D.M.S., Colonel C. H. K. Smith, M.C., for granting the opportunity to carry out this work, and to Kenneth Mellanby, Ph.D., for helpful criticism and advice.

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STUDIES ON THE POPULATION OF HEAD-LICE, *PEDICULUS HUMANUS* VAR. *CAPITIS* DE G.

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INTRODUCTION

The material for this study was obtained from 67 louse-infested patients, comprising 17 males and 50 females, who were all evacuees from Burma and were admitted for the treatment of other diseases in the Carmichael Hospital for Tropical Diseases in Calcutta during 1942. The evacuees after their arrival were temporarily housed in camps from where they were sent to different hospitals. More than half of those admitted in the Carmichael Hospital were Anglo-Indians, and the rest were Europeans, Americans, Anglo-Burmans, Burmans and Indians. The duration of the infestation did not exceed three months.

METHODS

For the study of the insect population infesting the hair Buxton (1936) introduced the method of dissolving the whole crop, removed by shaving, in an alkaline sulphide solution. The adoption of this method, however, was not possible in any of our cases. The specimens for our study were collected from a towel which was spread over the bed on which dead lice fell when the hair was combed following an application of a spray composed of pyrethrum extract and kerosene (Roy & Ghosh, 1942). A fine-toothed steel comb was used which was from time to time immersed in a bowl containing methylated spirit; this caused the lice sticking to the comb to become quickly dislodged. The combing was repeated daily till no more specimens were available.

OBSERVATIONS

The results of our studies are incorporated in Tables 1–3.

Every adult specimen was carefully examined and not a single individual showing signs of intersexuality, as reported by Keilin & Nuttall (1919), was encountered.

DISCUSSION

Studies on the distribution of lice on heads in different parts of the world were initiated by Buxton (1926, 1938, 1940, 1941). Of particular interest to us is his report on their distribution in Cannanore, south-western India, where collections were made from the inmates of the local jail (Buxton, 1940). Among those infested, two-thirds had from 1 to 10 lice and counts over 100 were rare, amounting to less than 3% of the infested heads, the highest count being 385 lice. According to this author the rate of infestation was high but the number of lice per infested head was low. In our series the highest count of adult lice, i.e. 1434, was from a short-haired female head (Table 2) and counts over 100 were made in 23 among 67 individuals amounting to 34·32% of infested heads (Table 2). In three, who were all females, counts over 500 were made. This gives a fair idea of the rapid multiplication of lice which may occur under the most favourable environmental conditions within a period of 3 months. It is necessary to state that none of these three patients had applied oil or had used a comb since they were infested.

The adult population was found to be at a low level in those cases who had attempted to remove the infestation by combing, washing and by applying oil to the hair. These measures did not either kill the adults or prevent the females from laying eggs.

Among the total adult population of lice collected by us there was a preponderance of one sex over the other (Table 1). Buxton (1941) also encountered an unequal distribution of the two sexes amongst his collections made from six different places. In collections from Cannanore the percentage of the male population was 46·4, or in other words there was 1·15 female per 1 male. Out of 8702 adults collected by us the male population comprised 23·34%, or 3·28 females per single male.

The average number of larvae* per female louse has been recorded in Table 3, the highest figure being 9.

5·06 was the average number of nits on a single hair weighing roughly 0·992 mg. The total number of nits present on a lock of hair removed from one head was calculated as 4288·3. Among these were 17% of nits still unhatched. The minimum distance of the first nit from the scalp was found to be 4 mm. which represented roughly the length of the body of a female louse. The longest distance of the last nit from the scalp was found to be 33·02 cm., and 11·7 cm. was the longest distance between two consecutive nits. These observations were made on the

* The three nymphal instars have been included under one heading, 'larva'.

Table 1. *Gross infestation rate of adult lice*

No. of heads		Adult lice: Total no.	Adult lice: ♂	Adult lice: ♀	Gross infestation rate per head	Maximum no. found on a single head
Male	17	8702	2031	6671	129·9	1434
Female	50					
	67					

Table 3. *Incidence of larvae in relation to female lice*

No. of heads		Total no. of female lice	Total no. of larvae	Average no. of larvae per female	Average no. of larvae per head
Male	17	880	1,376	1·5	80·9
Female	50	5791	23,335	4·02	466·7

Remarks. Eight heads, five male and three female, had adult lice but no larvae.

Table 2. *Distribution of adult lice on different heads*

	Numbers of adult lice																	
	1–10	Total no.	Males	11–50	Total no.	Males	51–100	Total no.	Males	101–500	Total no.	Males	501–1000	Total no.	Males	Above 1000	Total no.	Males
Long-haired heads:																		
Male	0	—	—	0	—	—	0	—	—	0	—	—	0	—	—	0	—	—
Female	3	18	4	7	246	56	2	160	47	4	957	238	0	—	—	0	—	—
Short-haired heads:																		
Male	7	28	2	5	85	17	0	—	—	5	1117	331	0	—	—	0	—	—
Female	6	21	4	9	273	70	5	366	78	11	2812	683	2	1185	252	1	1434	249
All heads	16	67	10	21	604	143	7	526	125	20	4886	1252	2	1185	252	1	1434	249

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hair of a very heavily infested female head who had not a single hair free from nits.

We would like to record here certain observations on the spread of lice from lousy to clean heads. On account of the rush of admission at that time verminous patients were mixed up with clean ones and both were kept in the same ward. Only the infested heads were treated in the ward soon after admission with a single application of a mixture of pyrethrum extract and kerosene or deobase oil and the patients were not allowed to wash their head within 24 hr. of treatment. Although the clean patients were not subjected to pyrethrum treatment there was no instance of such a patient becoming infested or of a patient after being deloused becoming reinfested.

The factor which prevented the population from establishing itself on the patient after treatment was probably that head lice cannot survive for more than 24 hr. if they are deprived of the opportunity of feeding. It seems doubtful if in nature they feed on any other part of the body except the scalp. It is also evident that stray lice were prevented from attacking the head of the treated patients on account of the presence of kerosene and pyrethrum on the hair which acted as repellents to these insects.

The reason why the infestation did not spread to any clean head was, according to us, the poor migratory habits of adults and larvae.

SUMMARY

The following is a summary of our studies on population of head lice, adults and nits.

1. Out of a total of 8702 adult lice, the male population comprised 2031. The gross infestation rate per head was 129·9 and the maximum number found on a single head was 1434. A disproportion in the distribution of the two sexes was a marked feature.

2. Patients below the age of 11 showed a lower infestation rate than those above this age, and short-haired individuals were more heavily infested than long-haired ones.

3. Frequent combing of the hair and the use of oil did not seem to dislodge the adults or to prevent the females from laying eggs.

4. The maximum larval count on a single head was found to be 4260, the average number of larvae per single head was 418·8, and 9 to 1 was the maximum proportion of larvae to females.

5. The maximum number of nits on a single hair was found to be 17 and 5·06 was calculated as the average number per hair.

6. Treatment with kerosene and pyrethrum killed all the lice and nits, and reinfestation did not occur.

7. The infestation did not spread to healthy patients in the hospital ward; it is thought that the poor migratory habits of lice are responsible for this.

8. From studies on the reinfestation of an individual after treatment it is concluded that head-lice cannot establish themselves on any part of the body except the head, and if their access to the head is prevented, the lice will not survive for more than 24 hr.

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COCCIDIAN OOCYSTS

By HELEN PIXELL GOODRICH, M.A. Oxon., D.Sc. Lond.

(With 6 Figures in the Text)

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COCCIDIA OF FOWL

As it was desirable to be able to determine the viability of oocysts in experimental work on fowl coccidiosis at the New Haw Veterinary Laboratory, Weybridge, I was asked by Mr Horton Smith to devise a suitable viability test which did not involve feeding cysts to susceptible chickens. He kindly sent me plentiful supplies of oocysts: these were chiefly *Eimeria tenella* (Fig. 1), but some of the other species of *Eimeria* from fowls were often in the suspensions together with some faecal debris.

Excystation of Eimeria tenella *in vitro*

Sporozoites have often been liberated from spores *in vivo* and also *in vitro* by mounting them in suitable media, that is, natural duodenal fluid or artificial mixtures containing trypsin (see p. 77), acting at the host's temperature and the normal *p*H of its duodenum. Some people profess to have obtained the sporozoites direct from oocysts, but the times given are very diverse, varying from 1 to 23 hr., and it seemed that most of the work had not been properly controlled with the microscope.

By careful microscopical study* it was soon evident that these differences in time were due to the condition of the oocyst walls, which are generally thick and impervious. Should they, however, be fractured accidentally, as often happens in nature (see p. 75 below), or intentionally, by tapping the cover-glass, then the ferment could get into contact with the spores. The sporozoites, if alive, would then start moving almost immediately and escape in a few minutes from the sporocyst (Fig. 1 *a*, *b*, *c*). When the oocyst walls were broken, some or all of the spores were generally set free, but, if only cracked, the ferment admitted could act on the spores and set free the sporozoites within the oocyst where they often remained for a considerable time, feeling for the crack with their extensible anterior ends and trying to wriggle through. In this they were, however, by no means always successful, and after some hours the movements would gradually cease. The sporocysts in these *Eimeria* are single and quite thin, closed by a disk-like stopper or operculum at the narrower end (Fig. 1 *o*). After this had been loosened and pushed out, the active sporozoites often took a few minutes to squeeze themselves through the minute terminal aperture. As a rule a residuum was left inside the sporocyst (Fig. 1 *a*), though occasionally it has been seen to be pushed out ahead of one of the sporozoites.

* For details of the practical methods for testing viability see Appendix.

Impermeability of oocysts

In all the species of *Eimeria* which I have examined the translucent oocyst walls have been double—the outer (ectocyst) being comparatively thick, rigid and often yellowish. Contrary to what has often been stated, the inner wall (endocyst) is the thinner and, if the outer one alone is fractured, an air bubble may enter between them causing a striking black refringence (Fig. 5). So resistant and impervious are these walls that Pérard (1925) and Wenyon (1926, p. 838) report having 'fixed', stained and mounted in balsam a quantity of zygotic oocysts, some of which completed their development up to the sporozoite stage on the slide, showing that none of the reagents had penetrated the oocyst walls.

This being so, one can hardly be surprised that oocysts may pass through the alimentary canal of their host intact and may also be kept in sterile

ferment solutions (pepsin and trypsin) for hours apparently unchanged. If, however, unduly detained in septic surroundings, such as the intestine or moist faeces, the oocyst walls tend to macerate and become limp, folded and misshapen, with bacteria often massed around them. The contained spores may be in various stages of necrosis; in the final one the sporozoites are reduced to a refringent mass inside the spore wall. Many of the intermediate stages are well represented in Robertson's figures of *Eimeria* (1933) from a dead hare showing the poor material on which he had to work. After prolonged maceration the necrotic spores themselves may merely form a refringent lining to part of the oocyst which then often appears to be empty.* On the contrary, I have obtained active living sporozoites from oocysts kept for more than 25 months in a suitable fluid (see below, p. 76). In fact, so far as I can make out, after studying thousands of oocysts of different species of *Eimeria*, unless the spores are freed in some mechanical way from the impervious ectocysts there is little chance of their being able to germinate.

In other Sporozoa, including the closely related Isospora, it is usual for the spores to be liberated from the cyst containing them either before passing out of the host, as in Aggregata (Pixell Goodrich, 1914, p. 170), or at any rate before being reingested. There may be, as in the genera *Gregarina* and *Nina* (Pixell Goodrich, 1938), elaborate processes of dehiscence; these are unusual, however, and there appears to be nothing of the sort in *Eimeria*, although some observers seem to have been so anxious to find a mode of dehiscence that they have even described the single residual globules in *E. tenella* and *E. ictidea* (Hoare, 1927) as 'micropyles'.

It was Metzner (1903) who first described the thin patch in the oocyst wall of *E. stiedae* as a micropyle 'opening to allow the escape of the sporozoites', and his figure of a sporozoite squeezing itself through this region has been copied into many text-books. Since normally this thin region is several times wider than a sporozoite, some investigation seemed advisable, and I proceeded to collect and examine rabbit coccidia. It was soon evident that the freed sporozoites were trying to squeeze themselves through cracks in the oocysts walls (Fig. 4) which may occur in any region and are often formed by partial drying as will be pointed out below (p. 76).

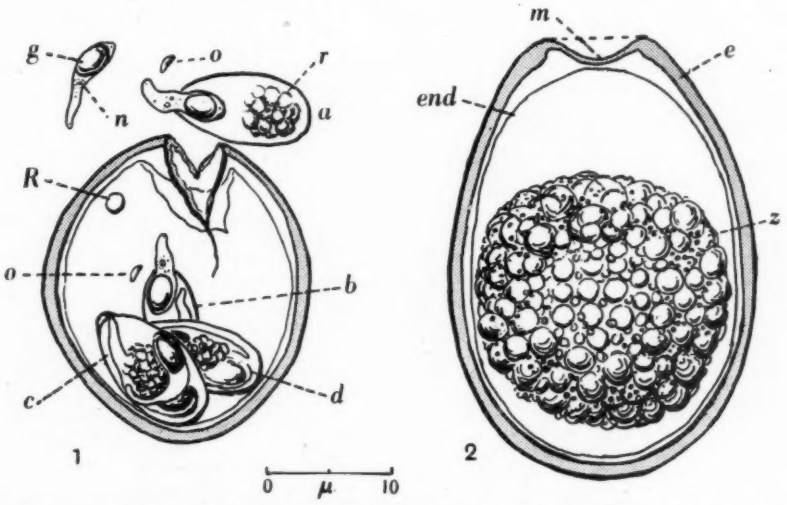


Fig. 1. *E. tenella*, ripe oocyst fractured and mounted in 5% trypsin solution at 37° C. Spore (*a*) free and opened with its second sporozoite escaping; (*b*) opened in ferment which has entered oocyst and one of its sporozoites protruding; (*c*) and (*d*) not yet opened, but in (*c*) movement has begun.

Fig. 2. *E. stiedae*, zygotic oocyst from gall bladder with concave micropylar region.

Lettering: *e*, ectocyst; *end*, endocyst; *g*, refringent globule; *m*, micropylar region; *n*, nucleus; *o*, operculum; *r*, residuum; *R*, residual globule; *z*, zygote.

* Krigsmann (1926) described as wrinkled and then empty oocysts which had been for 17–23 hr. in ferment at 37° C., and he apparently used this condition as a criterion of excystation and perhaps naturally found it more rapid after successive action of pepsin and trypsin.

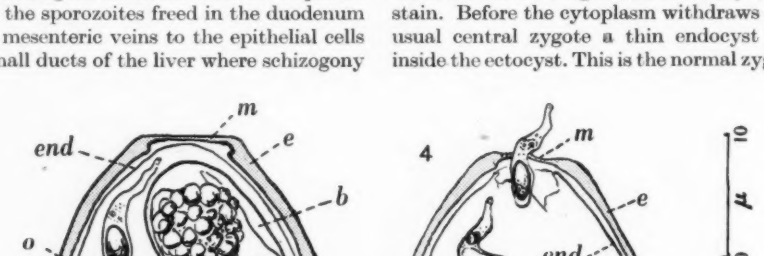
COCCIDIA OF RABBIT

Eimeria stiedae Lindemann is the best known of the

rabbit coccidia, being nearly always present in the liver of the common rabbit, *Oryctolagus cuniculus* L. Its synonomy and life history are given in most text-books, though it has been confused sometimes with other species occurring in the alimentary canal of the same host.

The figure given by Wenyon (1926, fig. 361 after Warworuntu, 1924) is not of *Eimeria stiedae* but of *E. magna*, with, as usual, a large oocystic residuum.

E. stiedae undergoes the whole of its development in the liver: the sporozoites freed in the duodenum pass by the mesenteric veins to the epithelial cells lining the small ducts of the liver where schizogony pass to the periphery where they flow together to form a continuous wall except for a minute hole, the micropyle, which is left for the entrance of the microgamete. After syngamy this hole becomes closed by some of the same secretion, but the region can still be distinguished in *E. stiedae* and some other species by the thinness of the wall, which is, however, thick enough to make the oocyst impervious to stains, and therefore easy to differentiate from the earlier stages which readily take up any stain. Before the cytoplasm withdraws to form the usual central zygote a thin endocyst is secreted inside the ectocyst. This is the normal zygotic oocyst



Figs. 3–6. *E. stiedae*, sporulated oocysts.

Fig. 3. Walls fractured by floating on saline and mounted in 5 % trypsin solution at 37° C. Two spores have fallen out. Of those left (*b*) is still intact and the two sporozoites (*spz.*) from (*a*) are trying to escape from oocyst.

Fig. 4. Fractured micropylar end of oocyst in trypsin solution; one of the two sporozoites, escaped from a spore, is trying to squeeze itself through a small crack.

Fig. 5. Micropylar region of oocyst fractured at one side after partial drying and two air bubbles (*x*) admitted. Mounted in distilled water.

Fig. 6. Micropylar region of intact oocyst, showing convex ectocyst after soaking in rain water.

(For lettering see Figs. 1 and 2.)

takes place for about 11 days (p. 78 below). After this the parasite is stimulated to undergo sexual reproduction and produces resistant stages (the oocysts) capable of living in the open and carrying the infection to other individual rabbits.

Structure and development of Eimeria stiedae *oocysts*

In sections through the liver of a rabbit, which has been infected for some weeks, macrogametocytes destined to develop into oocysts can be recognized in their host epithelial cells by the presence of secreted globules in the cytoplasm. These globules (Fig. 2), and, when it first falls into the lumen of the small duct, may have around it the remains of the exhausted host epithelial cell. This is, however, generally lost before the oocyst passes from the main bile duct into the duodenum and so through the rest of the alimentary canal to be evacuated. These zygotic oocysts may be found throughout the gut even at the distal end of the appendix and at times in the stomach owing to coprophagy practised by the animal (Taylor, 1940), but no further development can proceed at the temperature of the living host.

After evacuation, the zygotic oocysts will ripen

in the course of a few days, depending on the temperature. The optimum has been said to be 30° C. During this time the oocysts must be kept moist and free from bacteria and fungi (for suitable fluids see below, p. 76).

In the genus *Eimeria* ripening consists in the formation of four spores, each of which contains, besides a residual mass, two vermiform sporozoites for infecting another host. In some species, in addition to the four spores, there may be a residuum, as in *E. magna*, consisting of a mass of globules, or there may be only one (or occasionally two or three) as in *E. tenella*.

In the rabbit liver the number of parasites developing may be enormous, so that the ducts and gall bladder are crowded and the liver much hypertrophied and whitish: this condition is due to repeated infections.

If reinfection be prevented by moving an infected rabbit to a clean cage and site before its evacuated oocysts have time to ripen, then the primary infection may be localized to one or a few regions. When near the surface, these abscesses may appear on the red liver as opaque white spots or lines indicating surface ducts full of phagocyted parasites. There may even be no indication of infection on the surface, and the bile should be examined in order to be sure of the absence of previous infection.

By opening one of these abscesses in an otherwise healthy liver, many stages of the parasite in process of destruction are found amongst masses of phagocytes and debris. The globules of secretion which are ready to form the walls of developing oocysts are often seen collected in yellowish masses in which are sometimes embedded granules of cytoplasm and nuclear material from destroyed parasites. Some of this substance may attach itself to the ectocyst of those oocysts which have already managed to develop.

It is, of course, only those parasites which have undergone syngamy and have managed to close and complete their cysts which escape phagocytosis. To these the extra thickening by encrustation may even be of benefit, for it is often the thinner micropylar region that is covered by some of this liberated secretion forming a kind of cap, as drawn by Metzner (1903, Figs. 1–3) and others. Encrustations may occur on other regions of the oocyst, thus masking its usual shape, to some extent.*

The finding in faecal pellets of encrusted oocysts, which seem to have puzzled so many people, may be taken as an indication that the host is dealing satisfactorily with its infection, by phagocytosis.

Oocyst walls and their fracture

Besides these variable encrustations, the true ectocysts of *E. stiedae*, even from the same individual rabbit liver, do vary somewhat in thickness, as may be seen by mounting them in distilled water containing a dye readily absorbed by the walls. The staining reactions are similar to those of keratin: intense yellow with picric acid, pink with eosin, blue with many stains such as methylene blue and Stephen's ink. They stain like chromatin with haematoxylin, but not greenish, like chitin, with chlorozol black E; in fact, the clean ectocysts merely take a purplish tint after some time in this new dye (Cannon, 1937), but if there are traces of cytoplasm or nuclear material embedded in the encrustation attached to the outside these stain greyish or black.

The only coloured reagent which could be made to pass through all the walls of oocyst and spore was iodine (dissolved in water, alcohol, or potassium iodide solution), and that easily penetrates to the sporozoites, staining their large refringent oval bodies yellow and many granules deep brown (as also in *E. tenella*).

In solutions of thioglycollic acid* the ectocyst swells and gradually dissolves, making the endocyst very distinct. There was, however, neither with this nor any other reagent which I tried, the least sign of any opening. I am, in fact, quite convinced that the micropyle, closed as usual after syngamy, does not again open.

That the proteid of the ectocyst is allied to keratin† is indicated by its impermeability, resistance to weak acids and alkalis and especially its indigestibility by pepsin and trypsin. That it is not true keratin was shown, however, by the absence of birefringence with polarized light.‡

Normal unencrusted oocysts taken direct from the host and mounted in bile or normal saline have

* Cf. Wenyon's (1926, p. 846) '*E. canis*'. There would seem to be little doubt that the dogs in the faeces of which he found these parasites had been having an extensive feed on infected rabbits. All the oocysts given in his Fig. 365 might belong to one of the four or five species of *Eimeria* of the rabbit, and Wenyon himself mentions their resemblance to *E. stiedae* and *E. perforans*.

* Dr Philpot, of the Biochemical Department, kindly provided me with this and some other solutions.

† Kofoid, McNeil & Kopac (1931) concluded provisionally that the cysts of *E. histolytica* (and some other human intestinal parasites) had the properties of the group of keratins. So far as I know, no conclusive tests have yet been made on the single wall of *E. histolytica*. It is interesting to note that in *E. coli*, Dobell (1938) has stated that the endocyst, which is comparatively thick and rigid, resembles chitin in composition and that its opening (before that of the thinner and elastic ectocyst) is brought about by a ferment secreted by the enclosed Entamoeba and not by purely mechanical means.

‡ I am indebted to Mr P. 'Espinasse for demonstrating this and to Dr J. R. Baker for lending his microscope fitted with polaroid disks.

the appearance shown in Fig. 2, that is, the narrower end where the wall is thin (region of former micropyle) is indented to form a small crater. On focusing the edge of this crater the end of the oocyst appears flat, a condition which has often been described and which is maintained during ripening so long as the density of the fluid remains constant. The specific gravity of the oocysts in this state is approximately 1·09. If, however, they are soaked in distilled or rain water for some hours, the ectocyst, being slightly absorbent, swells and causes the crater to turn out and so form a convex end to the oocyst (Fig. 6); its specific gravity is then only about 1·088.

When transferred to saturated salt solution (1·21) the oocysts float, and not only does the micropylar end again become concave but other regions of the ectocyst also collapse and finally fracture. Since air may then easily enter between the walls, the characteristic black refringence is seen in many of these floating oocysts.

The ectocysts also collapse and fracture when oocysts are partially dried in air and the black refringence may appear long before the spores become necrotic; in fact, the air must be very dry to destroy them even hours after fracture of the ectocysts. The black refringent oocysts have frequently been examined in distilled water, and as they become soaked, the two walls show up clearly and are sometimes widely separated. It is then easy to see the cracks in the ectocysts, and the whole wall frequently chips off in pieces leaving the colourless endocyst containing the spores. The endocyst is sometimes perforated as the ectocyst breaks up, especially when oocysts with air bubbles between the walls are heated to body temperature. The endocyst is, however, so thin and flexible that it is generally pressed in round the spores and is probably broken almost directly after ingestion, if not before. I could not discover that either pepsin or trypsin solutions had any effect on the endocyst nor were free spores opened by the former, though as stated above they open rapidly in trypsin solutions.

Cracks, as already stated, may appear in any part of the ectocyst and naturally often occur near the edge or centre of the thin micropylar region (Figs. 4, 5). On one occasion a sporozoite freed by the entrance of the ferment was seen nipped in the very centre of this region and there held as in a vice while trying to escape: such accidents are, no doubt, the origin of Metzner's figure (see p. 73). There would seem to be little doubt that in nature alternate drying and moistening by rain or dew is likely to solve the difficulty of the impervious ectocyst.

Collection and ripening of oocysts

Zygotic oocysts for ripening may be collected from any region of the alimentary canal or from faecal pellets. If the infection is slight it may be necessary to concentrate the oocysts by screening, centrifuging or flotation. A pure culture of *E. stiedae* may be best obtained by removing oocysts from the gall bladder, washing them in water to free them from bile, and setting them to ripen in a suitable fluid which must prevent development of bacteria and fungi. If infected regions of the liver are placed in such a fluid after being cut up, the zygotes with closed oocysts will sporulate. If obtained from faecal pellets, the oocysts may be mixed with those of intestinal species.

The best moistening fluid for this purpose seems to be a dilute solution (about 2·5 %) of potassium bichromate or a 1 % solution of chromic acid. It is most important to prevent growth of both aerobic and anaerobic bacteria, but oxygen from the reagent does not seem to be necessary during sporulation, for a weak solution of such a reducing agent as formaldehyde will answer the purpose and so will many other antiseptics, such as thymol. Pérard (1924) used 5 % phenol and 1–10 % sulphuric acid and many other reagents without injuring the oocysts, but on the whole the chromium compounds seem to be best and, as they do not penetrate the walls, they can be washed away when necessary.

A few of the oocysts stored at ordinary room temperatures in these dilute solutions retained their viability for just over 25 months, but 2 years was the limit of life for most.

Becker (1934) quotes instances of survival for a year or more in a moist medium, free from bacterial action and at a temperature of 2–37° C.

For microscopical tests, oocysts were readily separated from faecal debris by a simple flotation method which eliminated the need for them to be in contact with the concentrated solution for more than a few minutes.

The specific gravity of most oocysts being approximately 1·09 they readily sink in water, bile and many solutions, even saturated $K_2Cr_2O_7$ (1·07), but will rapidly float up to the surface in such dense solutions as saturated salt (1·21). This inexpensive reagent answers the purpose admirably,* and in it most faecal debris and starch (1·53) remain sunk, while the oocysts float up and can be collected on clean cover-glasses dropped on to the surface. These can be transferred immediately to distilled water for washing before the concentrated salt solution has time to cause the walls to collapse and fracture. There can be no doubt, I think, that many of the strangely conflicting results recorded have been due

* In spite of this one finds many unsuitable reagents recommended—in one laboratory 30 % glucose was used. This has practically the same density (1·09) as the oocysts, and in it they would remain permanently suspended.

to prolonged action of saturated solutions acting on oocysts in bulk.

Ferments for opening spores

For testing the viability of oocysts (see Appendix) a dilute sterile solution of trypsin (B.D.H.), made slightly alkaline (*p*H 7·6) with 2% sodium bicarbonate solution, is satisfactory for opening the spores and liberating the sporozoites, if alive. I generally used a 5% solution but 0·5% was effective, and Medawar's* (1941) 0·5% trypsin mixture was often used. Natural duodenal fluid, aspirated from a freshly killed rabbit, was also very active.

At an early stage of this investigation, numerous oocysts in test-tubes, partly filled with the above trypsin mixtures, were incubated 1–18 hr. at 37° C. as described by Smetana (1933) for the excystation of *E. stiedae* oocysts. I always obtained negative results, unless some of the oocysts were purposely fractured before use, and so did Dr Francis Knowles who was helping me with the chicken coccidiosis, for a short time.

Smetana states that his material was ground in a mortar before ripening and collecting the oocysts in bulk by flotation. There can be no doubt that in this way many of his oocysts were fractured, and it was from these that he obtained his free sporozoites. Excystation, according to him, 'is first induced by the passive destruction or removal of the protein substance closing the micropyle'. If this were so, apart from what has been said above, it would entail a change in composition of the ectocyst between the first time it passes through the duodenum protecting the zygote and subsequent subjection, after sporulation, to the action of trypsin in the duodenum.

At times, many oocysts must be ingested before being fractured, and it was difficult to be sure whether they could be broken afterwards. At first sight it seems unlikely, knowing how difficult it is to break the walls of ordinary cells or bacteria mechanically, but wild rabbits swallow large quantities of sand, as do chickens, and it is possible that this might help to break the oocyst walls while passing through the specially muscular region of the pylorus or gizzard. To try to throw some light on this problem, the following experiments were undertaken to determine what actually does happen *in vivo* by studying the fate of sporulated oocysts fed to young animals.†

* I am grateful to Mr Medawar for providing me with quantities of this mixture and also with a 5% solution of trypsin in Tyrode's solution, specially made up. He also gave me the chicks used in the experiments described below, as soon as they were hatched in his laboratory.

† Mr J. Z. Young kindly conducted these few feeding experiments on chicks and rabbits under his licence.

EXPERIMENTS ON THE INGESTION OF OOCYSTS

(1) *In chickens*

Some freshly hatched chickens were given a varied diet for several days while their favourite food was noted. This proved to be Bemax, which was a suitable medium in which to give the oocysts and ensure their being swallowed.

An hour or so after its infective feed, the chick was chloroformed, and the contents of its duodenum, immediately behind the gizzard, were aspirated and examined in normal saline on a warm stage. Other regions of the gut were examined in a similar way.

(*a*) In a 7-day chick, killed 1 hr. after its infective feed, only one broken and empty oocyst case could be found in the anterior region of the duodenum and none behind this region, though there were many intact oocysts in the crop and gizzard also a few fractured ones still with four spores.

(*b*) In a 4-day chick fed 1¾ hr. before being sacrificed many oocysts were found to have reached the duodenum; a few of these were entire but most were broken and empty or with one or more sporocysts containing at most a residuum, i.e. sporozoites had escaped. After a prolonged search only a single oocyst (and that one entire) could be found in the rectum.

(*c*) On the other hand, in a 17-day chick killed 3 hr. after its infective feed, most of the oocysts appeared to have reached the rectum, and fractured ones were specially numerous near the entrance of the caeca, into which many sporozoites had already penetrated after escaping from freed spores. Some free sporozoites were also being evacuated together with many empty broken cysts and some entire sporulated ones.

In both birds *b* and *c* the gizzard had some empty broken cysts and also a few free spores. Their crops showed entire sporulated oocysts and on one occasion a broken one with only two spores left. Free spores have been recorded from the crop before.

(2) *In young rabbits*

Three young rabbits were fed with *E. stiedae* oocysts approximately 4 hr. before being killed. At the autopsies on the first two, the duodenal contents, aspirated immediately on opening, were found to contain no intact sporulated oocysts; one fractured one, containing four empty spore cases, was found in one rabbit. This was very surprising, and no oocysts at all appeared to be in the other parts of the intestine; they were, no doubt, still retained in the stomach which could not on this occasion be examined. In the third rabbit, however,

many were found in the stomach. Those in the pyloric region near the valve had fractured walls and some free spores were seen. In the duodenum a few free sporozoites were found, but again no intact sporulated oocysts.

All three of these rabbits already had slight coccidiosis, and the cardiac region of the stomach of the third contained zygotic oocysts due to coprophagy practised by the rabbit during the night or early morning (Taylor, 1940). This did not affect the results since oocysts never ripen in the body, the temperature being too high.

One other rabbit (T), known to be free from coccidia,* was, however, fed at intervals from 10 to 19 December with pure cultures of *E. stiedae* and specially fed at 6 and 9.20 a.m. before being killed at 10.35 a.m. on the 19th. The anterior end of the duodenum was at once fixed for sections which showed many fractured and empty oocysts in the lumen. In the stomach there were sporulated oocysts, and near the pyloric valve some broken ones and free spores were also seen.

From these experiments it can be concluded that ectocysts, enclosing spores destined to cause infection, are fractured before or during ingestion, and that the spores are set free near the pyloric valve (or in the gizzard of chickens) to open in the duodenum.

[Two other observations in connexion with the infection in this clean rabbit T should perhaps be briefly stated here.

(1) After the 9 days' ingestion of *E. stiedae* sections of the liver showed considerable infection of the epithelial cells of the small bile ducts, but only schizogonous stages were present, and no parasites could be found in the main bile ducts. This gives some confirmation of the finding by Smetana (1933) that gamogony does not start until the 11th day, and that the sporozoites find their way from the duodenum to the liver by the small veins.

(2) On 11 December the rabbit was given oocysts of *E. stiedae* and *E. magna*, the latter being an intestinal coccidian. On the 17th a few and on the 18th numerous oocysts of this species were evacuated. At autopsy on the 19th gamogony of *E. magna* was discovered to be proceeding in the epithelial cells of the duodenum in the region of the opening of the pancreatic duct, i.e. about 12 in. below the pyloric valve. In sections of this region the various sexual stages could be observed, but schizogony had ended—showing that this multiplicative process could only have taken place for 4 or 5 days, i.e. less than half the duration of schizogony of *E. stiedae* in the liver.]

* I am grateful to Prof. Sir H. W. Florey for this very healthy 4-week-old rabbit reared under specially clean conditions in the Pathological Department by Mr Jesse Wheal. The mother was also free from coccidia.

SUMMARY

1. The spores of *Eimeria* (not the oocysts) are the real infective agents and, as in other Sporozoa, they readily set free their sporozoites in the duodenum or *in vitro* under suitable conditions.

2. The protective oocyst has double walls: the ectocyst is thick and impervious to fluids but easily fractures by mechanical means, such as drying. Even though cracked, it may continue to protect the thin endocyst and spores until they reach the specially muscular regions of the alimentary canal. Free spores are probably also sometimes ingested.

3. The ectocyst is a continuous envelope without a persistent micropyle. It resembles keratin in many ways.

This work was done in the Department of Zoology and Comparative Anatomy, Oxford, and I am grateful to my husband, Prof. E. S. Goodrich, for help and advice.

APPENDIX

A quick method for testing the viability of ripe oocysts in normal saline solution.

A quantity of oocysts should be carefully mounted and examined while some of the fluid is evaporating on a warm stage.* If no fractured oocysts are present, a few taps on the cover-glass with a pencil or wooden handle will free numbers of spores.

The ferment† to be used should be run under the cover-glass at a temperature of 37° C. A small square of blotting paper at each side of the cover-glass will hold excess of ferment solution and prevent the preparation from drying up. The field will soon be covered with active sporozoites escaping from the spores.

Free spores unopened after 5 or 10 min. are necrotic, and the percentage of such spores may be calculated.

N.B. It is most important for these tests to have

(1) All slides and cover-glasses perfectly clean (free from grease).

(2) All oocysts well washed if they have been in antiseptics.

(3) A satisfactory warm stage fixed on the mechanical stage of the microscope. There is then no need to use a constant temperature room, though one at 37° C. is in many ways preferable.

For ordinary experimental work this test may be carried out with a dry high-power lens and should give quick and reliable results—with an oil-immersion lens and critical illumination an experienced observer can tell to some extent before adding the ferment whether a given oocyst contains spores with healthy sporozoites or ones too feeble to survive the struggle to escape, or whether they are already dead. A normal healthy sporozoite has

* Temperature 35–36° C. † Cf. p. 77.

a large refringent globule (Figs. 1, 3 *g*) filling up most of its hind region and behind it a terminal granule which probably has to do with secretion, since the posterior end is adhesive. Near the anterior end is also a granule specially well seen during extension. The nucleus, just anterior to the large globule, generally contains two refringent caryosomes.

In most preparations a few oocysts will be found which have not developed beyond the zygote stage or have developed abnormally. No difference could be discovered in oocysts of *E. stiedae*, whether obtained from the liver or faeces, nor was there any indication of change in the walls during sporulation. All appear to behave exactly alike and to fracture equally easily. The cracks may be very small or extend transversely or longitudinally right across the ectocyst.

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THE LUNGWORMS (NEMATODA: PSEUDALIIDAE) OF THE ODONTOCETI. PART I

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(With 13 Figures in the Text)

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INTRODUCTION

The nematode lungworms of the suborder Odontoceti (the toothed whales) belong to the superfamily Metastrongyloidea of the suborder Strongylina and constitute a group of genera whose species have, with two exceptions, been reported as occurring only in representatives of the family Delphinidae—porpoises and their close allies. The systematic treatment of these lungworms was largely inadequate until 1925. However, in that year Baylis & Daubney reviewed their systematics and morphology and placed them in four well-defined genera: *Pseudalius* Dujardin, 1844; *Stenurus* Dujardin, 1844; *Torynurus* Baylis & Daubney, 1925; and *Halocercus* Baylis & Daubney, 1925. Skriabin (1941) has recently mentioned two further genera, *Otophocoenurus* and *Skrjabinalius*, but I have been as yet unable to verify them, as he gave no citations to their original descriptions. (See Addendum.)

In two recent papers (Dougherty, 1943 *a*, *b*) I have reviewed the nomenclature of the lungworms of porpoises. For *Torynurus* Baylis & Daubney, 1925, the older name *Pharurus* Leuckart, 1848, has been substituted. In a third paper (Dougherty, 1943*c*) I have pointed out that two forms, which have been placed in the same genera as certain of the lungworms of porpoises but which themselves occur in carnivores, actually belong to the genus *Filaroides* v. Beneden, 1858. This genus is more closely related to certain other genera of lungworms in carnivores than to the genera in porpoises. The two forms thus transferred to *Filaroides* are *F. gymnurus* (Railliet, 1899) Dougherty, 1943 (syn. *Pseudalius*, or *Halocercus gymnurus*), and *kreisi* Dougherty, 1943 (syn. *Stenurus* sp., of Kreis, 1938).

It is now my intention to present observations on the general relationships of the lungworms of the Odontoceti and on the morphology of the individual species which I have been able to study first hand. Only a part of these observations appears in the present paper, which includes two sections, the first devoted to the relationships of the lungworms of porpoises and to the morphological evidence for my views, and the second to the genus *Halocercus* Baylis & Daubney, 1925, and its species.

Morphological terms and the classification used herein, except where change or innovation is noted, adhere to the usage of § I (on morphology) of an *Introduction to Nematology* by Chitwood & Chitwood *et al.* (1937–40). An effort has been made to associate the species of *Halocercus* with the taxonomically correct names of their hosts. I wish to express my appreciation to Dr Remington Kellogg, of the United States National Museum, for verifying the names of the porpoise hosts. I also wish to thank Dr Harold Kirby, of the Department of Zoology, for advice and criticism during the course of this work.

1. THE RELATIONSHIPS OF THE FAMILY PSEUDALIIDAE RAILLIET, 1916

I regard the lungworms of porpoises as a distinct family, Pseudaliidae Railliet, 1916. Baylis & Daubney (1925) have expressed the opinion that the pseudaliids are closely related to the lungworms of terrestrial mammals and consequently placed them in the family Metastrongylidae Leiper, 1909. It is such a group—including strongyline lungworms of both terrestrial and aquatic mammals—that has of recent years been generally accorded superfamilial rank. Baylis & Daubney doubted that sufficient differences exist to justify recognition of any grouping separating the lungworms of terrestrial mammals from those of porpoises. I believe, however, that certain considerations may justify a modification of this view. It is true that the latter lungworms approach certain of the former very closely. However, it should be noted that in the

configuration of the female reproductive system such genera as *Dictyocaulus* Railliet & Henry, 1907, and *Crenosoma* Molin, 1861, approach an organization typical of members of the family Trichostrongylidae Leiper, 1912, whereas such genera as *Metastrongylus* Molin, 1861, *Protostrongylus* Kamenskiĭ, 1905, *Filaroides* v. Beneden, 1858, and those in porpoises have a unique type of organization not found elsewhere in the Strongylina. I regard the organization of the female reproductive tract in this suborder as of considerable evolutionary significance.

Chitwood & Chitwood (1940) have discussed in detail the structure and terminology of the female reproductive system in the phylum Nematoda. They have pointed out the inconsistent use, by various writers, of names applying to its terminal parts and have selected certain standard terms. They have called the entire unpaired terminal segment of the female reproductive tract of nematodes the *vagina*, that part of ectodermal origin being designated the *vagina vera* and that part derived from the genital primordium, hence mesodermal, the *vagina uterina*. I have studied these structures in representatives of almost all the major groups in the three superfamilies of the Strongylina. The vagina vera in this suborder is of variable length and is in most cases directly preceded by two uteri, so that the vagina uterina is very short or non-existent. This is true of all members of the superfamilies Strongyloidea Weinland, 1858 (whether amphidelphic or prodelphic) and Trichostrongyloidea Cram, 1927 (although the heligmosomes may lose one uterus), and of certain forms currently held to be members of the superfamily Metastrongyloidea Lane, 1917. I have found, however, that a group of metastrongyloids, including the type genus of the superfamily, namely, *Metastrongylus* Molin, 1861, presents a striking deviation from the pattern already mentioned, for all members of this latter group possess a long vagina uterina.

In Fig. 1 the terminal parts of the female reproductive tracts of *Halocercus invaginatus* (Pseudaliidae) and *Trichostrongylus colubriformis* (Trichostrongylidae) are pictured. Further discussion may be primarily referred to these two forms. In the former species the two uteri join to form a vagina which opens through a vulva just in front of the anus; in the latter, the two uteri join to open through a vulva situated in the mid-region of the body.

I have interpreted the vagina vera in the genus *Halocercus* Baylis & Daubney, 1925, to include the vulvar lips and immediately adjacent tissue, which are set off quite sharply from the immediately preceding muscular segment of the reproductive tract. This muscular segment and the rest of the vagina thus constitute the vagina uterina—all of it being fundamentally uterine tissue despite regional specialization. Fundamentally there appear to be three regions: a proximal muscular part extending a short distance into the unspecialized paired uteri which join to form the vagina; then a more or less unmodified segment, the walls of which are only slightly different from those of the paired uteri; and finally the terminal, rather strongly muscular segment already mentioned. In the genus *Trichostrongylus* Looss, 1905, however, it is the ends of the uteri that are specialized, and these communicate directly with the vagina vera, so that no clearly marked vagina uterina is present.

To indicate function in the strongyline female reproductive tract Chitwood & Chitwood have employed certain terms adapted from the earlier usage of Looss, Seurat, and others. Where the vagina or the terminal parts of the uteri are specialized as in the Strongylina to facilitate egg laying or the expulsion of larvae, the term *ovejector* is employed. There are two ovejectors in such amphidelphic strongylines as the trichostrongyloids, ancylostomatids, and diaphanocephalids, and in both prodelphic and amphidelphic strongylids and in syngamids. In each ovejector of the first two groups there have been three regions recognized; these are the *infundibulum*, which receives the eggs or larvae from the unspecialized part of the corresponding uterus; next the *sphincter*, which as its name implies is a muscular segment; and finally the *ejector*, the terminal segment which conducts the eggs or larvae to the vagina. In strongyloids other than the ancylostomatids there is no clear division between the sphincter and ejector, which are entirely surrounded by a muscular sheath, but the infundibulum is always evident because of its partial or complete freedom from this sheath, its cells differing only in size from those of the uteri proper. In all these forms, however, the ovejectors do not constitute a part of the vagina uterina, as they are paired.

Superficially this difference between what may be termed the typical organization of the strongyline ovejectoral apparatus and that exhibited by the metastrongyloid group in which the vulva lies immediately in front of the anus is thus very great. However, it seems probable that the single ovejector in *Halocercus* is actually homologous with the pair of ovejectors in *Trichostrongylus*. Terms already applied to the ovejector in the former genus tend toward confusion. Baylis & Daubney referred to the terminal muscular segment of the vagina uterina as the 'vagina', and indicated that a well-developed 'sphincter muscle' sometimes surrounded it. The unmodified part of the vagina uterina they termed the 'common trunk of the uterus' and described it as a 'reservoir for embryos'. They did not note the more proximal muscular segment. Wu (1929) followed this usage. Lins de Almeida (1933 *a*, *b*), on

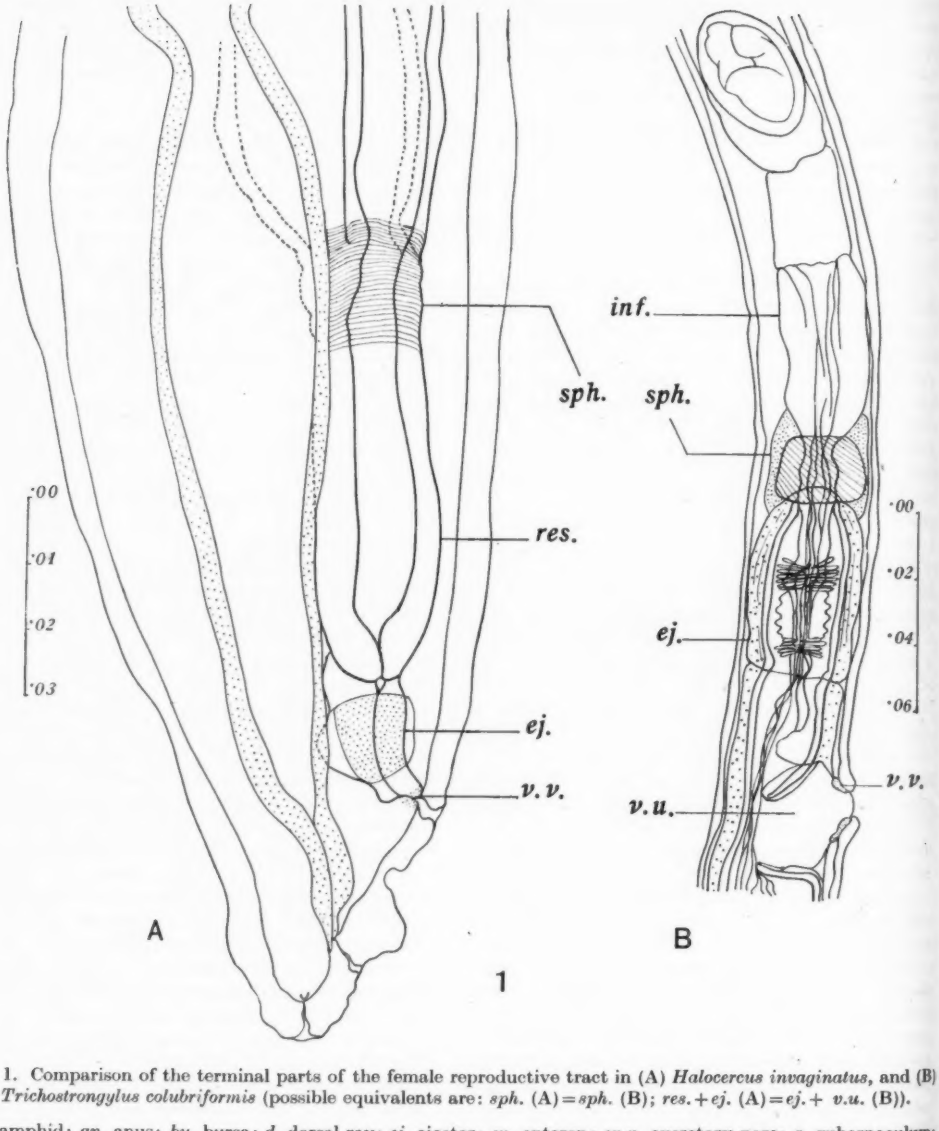


Fig. 1. Comparison of the terminal parts of the female reproductive tract in (A) *Halocercus invaginatus*, and (B) *Trichostrongylus colubriformis* (possible equivalents are: *sph.* (A)=*sph.* (B); *res.*+*ej.* (A)=*ej.*+ *v.u.* (B)).

am. amphid; *an.* anus; *bu.* bursa; *d.* dorsal ray; *ej.* ejector; *en.* enteron; *ex.p.* excretory pore; *g.* gubernaculum; *gl.* subventral gland; *inf.* infundibulum; *l.* lateral ray; *la.* larva; *li.* lip; *n.r.* nerve ring; *oe.* oesophagus; *ph.* phasmid; *res.* reservoir; *sp.* spicule; *sph.* sphincter; *sp.sh.* spicular sheath; *v.* ventral ray; *v.u.* vagina uterina; *vu.* vulva; *v.v.* vagina vera.

the other hand, although he employed the term 'vagina' similarly, designated Baylis & Daubney's 'common trunk of the uterus' as the 'ovejector'. The homologies of the unpaired ovejector in the Pseudaliidae and other metastrongyloids with the paired ovejectors of the rest of the Strongylina could be well established by means of histological comparison of the cells making up these parts and the mapping of their distribution. It is obvious without such a study that a rather considerable

difference in organization exists between the two groups. Furthermore, certain basic facts can be illustrated from gross study. In the Pseudaliidae and other metastrongyloids with pre-anal vulvae the paired ejectors of the Trichostrongyloidea must be represented by the unpaired portion called by Baylis & Daubney the common trunk of the uterus. The weakly muscular junction between the uteri and the vagina uterina in *Halocercus* might represent the sphincters of the trichostrongyloid ovejectors, but in that case the latter structures have partially fused, or specialized muscular tissue has extended to include part of the adjacent vagina uterina. Except for these muscular junctions the ends of the uteri are not visibly modified in *Halocercus*, and hence infundibula as seen among most strongylines are not distinguishable. However, I have seen two infundibula clearly in species of metastrongyloid genera closely related to *Halocercus*. But functionally these parts in the Pseudaliidae do not correspond exactly to possibly homologous parts in the Trichostrongyloidea, and in any event I do not wish to imply derivation of the former type of organization from the latter, although their ultimate common origin in the ancestry of the Strongylina is manifest. Since the names of the parts of the trichostrongyloid system are terms designed to express function, not homology, it would appear desirable to me in the interests of good and widely applicable usage to adapt this terminology to functionally analogous pseudaliid structures. Thus the muscular junction between uteri and vagina uterina may be termed the *sphincter*; the relatively unmodified part of the vagina uterina, the *reservoir*, as it serves to store embryos; and the muscular terminus, the *ejector*. The entire vagina uterina, including sphincter, reservoir, and ejector, thus makes up the ovejector.

It therefore appears that despite a rather striking difference in appearance the ovejector of the specialized metastrongyloid group, including the genera in porpoises, probably adheres to a basic strongyline pattern. It seems logical, however, to regard the deviation from a more general organization of paired ovejectors as a significant morphological departure and thus as of considerable taxonomic significance. The type of unpaired ovejectors exhibited by *Halocercus* is shared by the other genera of metastrongyloids in porpoises. In related genera from terrestrial mammals, however, no terminal muscularization of the vagina uterina is obvious, and thus an ejector is lacking. The entire structure except for the muscularized sphincteral region may therefore be termed a reservoir.

I do not wish to propose at this time any major changes in strongyline classification nor to present a detailed reclassification of the strongyline lungworms of mammals. It is sufficient to suggest a tentative grouping of the latter (except *Syngamus* v. Siebold, 1836) into three families, distinguished by the nature of the ovejectoral apparatus of the female: (1) Dictyocaulidae Skriabin, 1941 (with paired trichostrongyloid ovejectors), (2) Metastrongylidae Leiper, 1909 (unpaired ovejector, without an ejector), and (3) Pseudaliidae Railliet, 1916 (unpaired ovejector, with an ejector). It is interesting to note that Skriabin (1941), for biological instead of morphological reasons, referred his family Dictyocaulidae (including only *Dictyocaulus*, however—not *Crenosoma* or its allies which I refer to this family) to the superfamily Trichostrongyloidea.

I believe that the family Pseudaliidae can be further distinguished from other possible metastrongyloid groups by the fact that the bursa of the pseudaliid male has been considerably reduced from the typical strongyline condition, and the thirteen original rays have been fused into five atypical rays, paired ventral and lateral, and unpaired dorsal, the externodorsals of the generalized bursa apparently having fused with the lateral rays. In the Metastrongylidae and Dictyocaulidae such reduction of the bursa and fusion of the rays as has occurred has followed essentially different patterns. The genus *Filaroides* and its relatives, placed by several authors in the Pseudaliidae as a separate subfamily, Filaroidinae Skriabin, 1933, are more closely related to the genus *Metastrongylus* and certain other metastrongyloids of terrestrial mammals and belong to the family Metastrongylidae.

II. THE GENUS *HALOCERCUS* Baylis & Daubney, 1925

Phylum NEMATODA*

Class PHASMIDEA

Order RHABDITIDA: Suborder STRONGYLINA

Superfamily METASTRONGYLOIDEA

Family PSEUDALIIDAE

Genus **Halocercus** Baylis & Daubney, 1925

Synonymy: *Strongylus* Müller, 1780 (*partim*). *Filaria* Müller, 1787 (*partim*). *Pseudalius* Dujardin, 1844 (*partim*).

Diagnosis. Long, thread-like worms with a tendency toward cuticular inflation over part of the body. Posterior end of male somewhat conical, of female truncate or conical. Phasmids present as tiny papillae on female posterior end. Oral opening small; stoma rudimentary; lips inadequately de-

* Chitwood's use (1940) of Nemata, an emendation of Nemates Cobb, 1919, seems an unnecessary change. I prefer to retain for the phylum the name Nematoda Diesing, 1861, originally used as an order.

scribed for most species, but presumably of a similar pattern in all, i.e. six in number, two lateral, two subdorsal, and two subventral, bearing an internal circle of six cephalic papillae and an external circle of ten cephalic papillae and the amphids. Oesophagus cylindroid. Deirids not observed. Excretory pore and subventral glands presumably well developed in all species, the former opening at the level of the posterior end of the oesophagus. Male genital system: bursa rudimentary and rays extremely reduced, ventral ray with a very large base and a single (or possibly in *H. brasiliensis* a double) termination, lateral rays with three terminations, at least in some species, although reported with two in others, dorsal ray with two blunt terminations; spicules basically slender and arcuate, but variously modified, unfused at tips; gubernaculum moderately well to poorly sclerotized, or apparently absent. Female genital system: vulva with distinct non-cuticular lips which have no cuticular ornamentations and apparently constitute, with a small amount of adjacent tissue, the vagina vera; ovejector with a weakly developed sphincter at end of uteri, an unpaired narrow to wide reservoir filled with embryos, and a terminal muscular ejector; two genital tubes, each of which runs a complicated course, one being confined to the posterior half of the body, the other extending into and largely confined, except for the terminal portion, to the anterior half of the body.

Genotype. Halocercus delphini Baylis & Daubney, 1925.

Discussion

Halocercus was established by Baylis & Daubney (1925) to receive two well-defined species of nematodes from the bronchi of porpoises, *H. delphini* (genotype) from the common porpoise (*Delphinus delphis*) and *Halocercus lagenorhynchi* from the white-snouted porpoise (*Lagenorhyncus albirostris*); to the genus these authors also tentatively assigned, as *Halocercus gymnurus*, a nematode described by Railliet (1899) from the bronchioles and parenchyma of the lungs of the harbour seal (*Phoca v. vitulina*), and in an addendum they included, as *Halocercus inflexocaudatus*, a fourth species described by von Siebold (1842) from cysts in the parenchyma of the harbour porpoise (*Phocoena phocoena*). Since the publication of Baylis & Daubney's paper there have been described from porpoises two additional species, *Halocercus pingi* by Wu (1929) from spaces in the parenchyma of the lungs of the Chinese black finless porpoise (*Meomeris phocaenoides*) and *Halocercus brasiliensis* by Lins de Almeida (1933*a*) from the bronchi of the Brazilian fresh-water porpoise (*Sotalia brasiliensis*); the genus itself has been reviewed by Lins de Almeida (1933*b*); species occurring in porpoises of the North and Baltic Seas have been recorded by Schuurmans Stekhoven (1935); Schmidt-Ries (1939) has redescribed under the name of *Halocercus inflexocaudatus* the species from the harbour porpoise; and another, hitherto unnamed species has been recorded by Benson & Groody (1942) and by myself (Dougherty, 1943*a*) from nodules in the lungs of a specimen of the Dall porpoise (*Phocoenoides dalli*). In recent papers (Dougherty, 1943 *a*, *b*) I have indicated certain changes in the nomenclature of porpoise lungworms, and in a third paper (Dougherty, 1943*c*) I have suggested changes in the nomenclature of forms from hosts other than porpoises, but previously placed in porpoise lungworm genera. Among the species discussed in the first and third of these papers were two of the genus *Halocercus*. The form called *H. inflexocaudatus* (from the harbour porpoise) by Baylis & Daubney and others has been redesignated *H. invaginatus* in the first; and the form known as *H. gymnurus* (from the harbour seal) has been transferred in the third paper from *Halocercus* to the genus *Filaroides* v. Beneden, 1858. (See Addendum for further species.)

I wish here to analyse some of the previous literature on *Halocercus* and its species and to present a revised diagnosis of *H. invaginatus* and a description of the new species from the Dall porpoise. The latter was discovered by Dr Harold Kirby of the University of California, Department of Zoology, and is therefore named *Halocercus kirbyi* n.sp.

In the diagnoses of *Halocercus* and its species I have employed terms applying to structures not previously recognized in pseudaliids and have introduced certain substitute terms for already known structures. Thus in *Halocercus* the *amphids* and *phasmids* have not hitherto been recognized in their true nature; the term *stoma* has been substituted for 'buccal capsule'. The nomenclature of the female reproductive tract has already been discussed.

In studying *H. invaginatus* and *H. kirbyi* I have concerned myself with certain morphological details that have been imperfectly described or even undescribed for other species. Some of these observations are doubtless applicable to all species of the genus.

The disposition of the female reproductive tract is of interest. In describing *H. delphini* Baylis & Daubney stated that the 'female genital tubes run forward to about the junction of the anterior and middle thirds of the body'. Lins de Almeida (1933 *a*, *b*) indicated that in *H. brasiliensis* the ovaries extend just anterior to middle of the body. No data have been given for *H. lagenorhynchi*, *H. pingi*, or *H. invaginatus*. I have found that the same arrangement of the uteri and ovaries exists in both *H. invaginatus* and *H. kirbyi*, and I assume that a similar condition is true of the other species. If I am correct in this assumption, Baylis & Daubney's and Lins de Almeida's observations re-

quire emendation. In the two species studied, I have found that the genital tubes are prodelphic, but that one is reflexed from slightly more to slightly less than half-way from the anterior end, whereas the other extends on anteriorly. It has not been possible for me to determine of which tube this is true in sufficient cases to establish any definite rule, as both tubes are wound about the intestine and in most of my specimens cannot be followed individually for their entire length. The reflexed tube runs back to within one to a few millimetres from the posterior end and then turns anteriorly to end before it reaches the level of primary reflexion. The other tube runs forward to a point from slightly less than half to about one-fifth the body length from the anterior end, is there reflexed, runs back a varying distance, sometimes as far as the middle of the body, again turns anteriorly, runs to a point from about one-third to less than a fifth of the body length from the anterior end, and there turns posteriorly to end after a rather sinuous course before reaching the level of the posterior uterus, in some cases even turning anteriorly again for a short distance. Thus, fundamentally, one uterus is restricted to about the posterior half of the body, whereas the other undergoes its flexions in the anterior half of the body. Possibly this condition is to be interpreted as a survival of a more primitive stage in the disposition of the female reproductive system, when the vulva was equatorial in position and the uteri amphidelphic. The disposition of the genital tubes in the female of *H. invaginatus* is pictured in Fig. 2. Thus the statements by Baylis & Daubney and by Lins de Almeida concerning the most anterior extent of the female reproductive tract doubtless refer to the more anterior genital tube.

The bursa in *Halocercus* is greatly reduced or even indistinguishable from the general body cuticle. The bursal rays are so degenerate as scarcely to need any cuticular support. The number of their terminations is very likely the same throughout *Halocercus*. Only one termination has been observed by me on the ventral ray in the two species studied or has been reported for other species in the genus, with the exception of *H. brasiliensis* as described by Lins de Almeida. Rejection of the latter's observation is, however, impossible without study of original specimens of his species; but it may be noted that, where a less vestigial ventral ray exists, as in *Pharurus*, in which the two ventral rays of the normal strongyline bursa—lateroventral and ventroventral—are apparent, although fused, the terminations are very close together, whereas the two pictured by Lins de Almeida are quite separate. I have observed three terminations on the lateral rays of *Halocercus invaginatus* and *H. kirbyi*, while only two have been reported by workers on the other species. To what degree the ray terminations correspond to the nine pairs of nerve endings and the paired phasmids basic to the posterior end of the rhabditine male, it is impossible to be sure. No prebursal papillae are in evidence. Possibly in rays as reduced as in *Halocercus* partial fusion or loss of nerve endings has occurred. Presumably, if this is

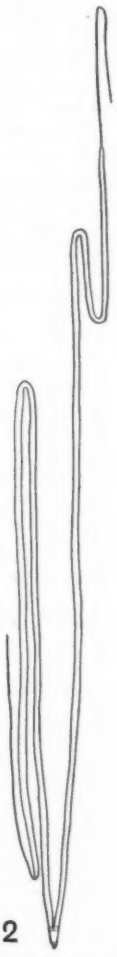


Fig. 2. The disposition of the genital tubes in *Halocercus invaginatus*; diagrammatic.

not so, then there must be a double innervation of each ventral ray and at least a triple innervation of each lateral; a fourth innervation of the latter should also probably be sought inasmuch as the externodorsal rays of the normal strongyline bursa apparently constitute part of the lateral rays in the Pseudaliidae. The dorsal ray should have six terminations, unless some of these have moved an-

teriorly on to the body proper. In *H. kirbyi* there seem to be a few tiny papillae just posterior to the cloacal opening, but the actual existence—not to mention the number and exact distribution—of these could not be definitely ascertained in my material. Possibly these papillae exist and represent nerve endings which have migrated off the dorsal ray during the regressive evolution of the bursa in *Halocercus*.

One character of some importance is the pattern of cephalic papillae. It has appeared to me that in the *en face* view of the head of *H. kirbyi* the six lips ranged about the mouth bear both the internal and external papillary circles (as indicated in Fig. 11). It can be definitely asserted that the amphids open on the lips. But the general papillary distribution is so difficult of determination even under the oil immersion objective that I acknowledge my interpretation to be in part subjective. A similar pattern seems to be possessed by *H. invaginatus*, but because of a greater delicacy of its lips and papillae a still larger degree of subjectivity was demanded in the interpretation thereof. Conclusive evidence can probably best be obtained with proper fixation and sectioning of the anterior end of one of these worms. If substantiated, this condition in which both cephalic papillary circles are on the lips is apparently unique in the Metastrongyloidea.

The several species of *Halocercus*, except *H. lagenorhynchi* and *H. pingi*, are easily distinguished on the basis of their spicules, which are fundamentally arcuate, but rather variable in morphology—in the development of alae and in other particulars. In general the spicular lamina is easily recognizable by its alae, but the calamus and capitulum are not distinctly marked off from one another. The spicules of *H. delphini* are more or less rodlike, whereas those of *H. lagenorhynchi* and *H. pingi* have quite wide, rather thin alae, which taper off distally. Those of *H. brasiliensis* each have a bifid tip, are tubular, and have a swelling a short distance from the proximal end. *H. invaginatus* and *H. kirbyi* have much shorter spicules. The spicules of the former somewhat resemble those of *H. delphini*, whereas in the strong development of the alae the spicules of *H. kirbyi* are like those of *H. lagenorhynchi* and *H. pingi*. There are, however, in *H. kirbyi* conspicuous alar expansions along either side in the middle of each spicule, whereas in *H. lagenorhynchi* and *H. pingi* there is no such development. Five of the six species of *Halocercus* are undeniably distinct. There is some question in my mind as to the validity of *H. pingi*, which is quite possibly a synonym of *H. lagenorhynchi*. However, in absence of positive evidence for this identity I prefer to leave them separate species. The resulting six species are each restricted to a single host species, with no host harbouring more than one species of *Halocercus*.

Table 1. *Principal measurements in mm. of the species of* Halocercus

Measurements	*H. delphini*	*H. lagenorhynchi*	*H. pingi*	*H. brasiliensis*	*H. invaginatus*	*H. kirbyi*
Length: ♂	50–65	—	150–183	30–35	8–23	30–42
♀	60–90	93+	255–364	45–52	17–65	52–88
Width: ♂	0·43*	0·34*	0·45–0·60	0·39–0·42	0·17† (0·25 mm., Schmidt-Ries)‡	0·125*
♀	0·5*	0·4*	0·55–0·68	0·47–0·60	0·17† (0·4 mm., Schmidt-Ries)‡	0·150*
Oesophagus: Length	0·6	0·2	0·19–0·22	0·22–0·28	0·105–0·195	0·17–0·21
Width	0·03	—	0·035–0·039	0·028–0·05	0·010–0·025	0·016–0·025
Subventral glands, length	—	—	Approx. 1–1·5	0·84–1·1	0·290–0·695	—
Spicules, length	0·73–0·76	0·65	0·77–0·82	0·6–0·7	0·150–0·205	0·125–0·145
Vagina vera + ovejector	0·12 (?)	0·63	0·65–0·87	0·44–0·60	0·15–0·35	0·305–0·615
Vulva to anus	—	0·088	—	0·04–0·056	0·031–0·063	0·021–0·038
Vulva to posterior end	—	—	—	0·07–0·096	0·048–0·100	0·036–0·069

* Maximum dimension. † Without cuticle. ‡ ? With cuticle.

Measurements of the six species of *Halocercus* are presented comparatively in Table 1, and the following key is offered for practical specific determination.

1. Spicules more than 0·6 mm. long. 2
   Spicules less than 0·25 mm. long. 5
2. Spicules each with a bifid distal end and a swelling a short distance from the proximal end. **H. brasiliensis**
   Spicules without bifid distal ends or proximal swellings. 3
3. Spicules more or less simply rodlike with inconspicuous alae. **H. delphini**
   Spicules with conspicuous, thin alae. 4
4. Gubernaculum with a double fold. **H. lagenorhynchi**
   Gubernaculum with a triple coil. **H. pingi**
5. Spicules more or less simply rodlike. **H. invaginatus**
   Spicules trough-like with conspicuous alae strongly expanded in middle of spicule **H. kirbyi**

Halocercus invaginatus (Quekett, 1841) Dougherty, 1943

(Figs. 1–8)

Synonymy: *Strongylus invaginatus* Quekett, 1841,* p. 151. *Filaria inflexocaudata* v. Siebold, 1842, p. 348. *Pseudalius tumidus* Schneider, 1866, p. 174. *Filaria inflexicaudata* Cobbold, 1879, pp. 425, 430 (*err. pro Filaria inflexocaudata*). '*Filaria*' *inflexocaudata* v. Siebold, 1842, of Baylis & Daubney, 1925, pp. 213–14. *Halocercus inflexocaudatus* (v. Siebold, 1842) Baylis & Daubney, 1925, pp. 215–16. *Halocercus invaginatus* (Quekett, 1841) Dougherty, 1943*a*, pp. 19, 20.

Diagnosis.† Length, male 8–17 mm., female 17–31 mm. Maximam thickness (without cuticle), male 0·170 mm., female 0·170 mm. Cuticle strongly and irregularly inflated over entire body. Male posterior end bent ventrally. Oral opening equipped with six small lips set back from mouth. Oesophagus very short; length, male 0·105–0·140 mm., female 0·120–0·165 mm.; maximum width, male 0·010–0·017 mm., female 0·010–0·019 mm. Nerve ring at about two-thirds length of oesophagus from anterior end. Excretory pore opening at the level of the posterior end of the oesophagus; subventral glands unequal in length, the shorter 0·290–0·600 mm., the longer 0·320–0·695 mm. Male genital system: cuticle of bursa indistinguishable from body cuticle; ventral rays with one termination on a thick base, lateral ray with three terminations, two slender and digitiform, one short and blunt, dorsal ray with two blunt terminations; spicules very short, simply arcuate, with distinct sheaths, each spicule divisible into a proximal capitulum, a slight constriction marking the calamus, and a curved lamina, 0·150–0·205 mm. long; gubernaculum rather weakly sclerotized, 0·025–0·045 mm. long; testis extends forward to about one-third of body length from anterior end. Female genital system: vulva with distinct lips, 0·031–0·063 mm. from anus and 0·048–0·088 mm. from posterior end; combined length of vagina vera and ovejector 0·150–0·225 mm., ejector 0·031–0·075 mm. long, reservoir with a single row of embryos; anterior ovary extends forward to within one-fifth to one-third of the body length from the anterior end.

Type specimens: presumably lost. Specimens from present study: some deposited in the United States National Museum (U.S. Nat. Mus., Helm. Coll. No. 36876); others sent to the British Museum (Natural History) in London, to the Instituto Oswaldo Cruz in Rio de Janeiro, and to the Vsesoĭuznyĭ Institut Gel'mintologii in Moscow.

* October 1841, not 1842 as Baylis & Daubney and other authors have indicated, nor November 1841, as v. Siebold (1842) indicated.

† Based on specimens in present study.

Type and only host. Phocoena phocoena (Linné), harbour porpoise.

Habitat. Tiny cysts scattered throughout the lung parenchyma.

Geographical distribution. Type locality: probably waters of the British Isles (not stated by Quekett). Other localities: Baltic Sea (Schneider, 1866; Schmidt-Ries, 1939); San Francisco Bay, California, U.S.A. (Dougherty, 1943*a*); Neah Bay, Washington, U.S.A. (new record*).

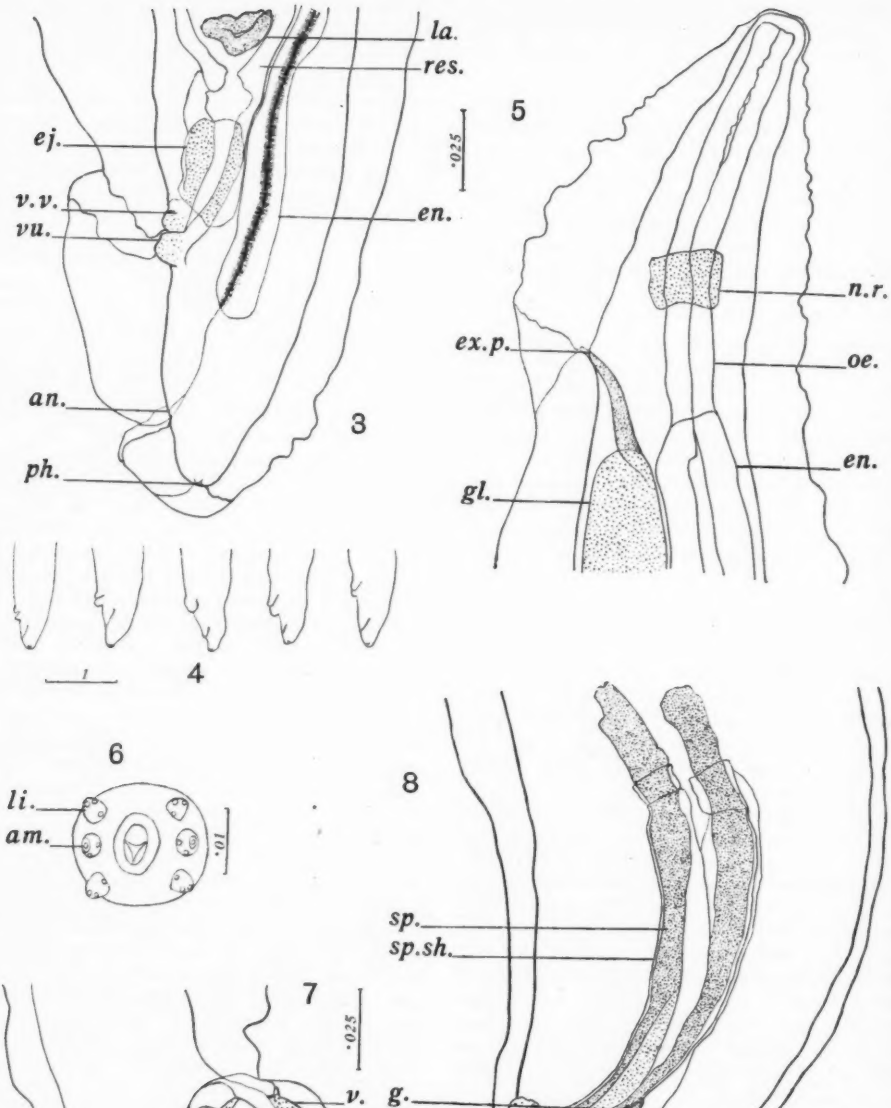
Discussion

Halocercus invaginatus seems to have been first seen by Craigie (1832), who, however, evidently confused it with an entirely different and much larger worm, *Pseudalius inflexus* (Rudolphi, 1808) Schneider, 1866. Though, if one may judge by the descriptions, characteristic cysts in the parenchyma of the lungs were seen by Craigie and by Eschricht (1841 *c*, *d*), the worms coiled up within them were thought to be a young stage of *P. inflexus*, and Quekett (1841) was the first to recognize them as a distinct species. Von Siebold (1842), in ignorance of Quekett's work, gave to the same worm the name of *Filaria inflexocaudata*, and it was again described as new by Schneider (1866) under the name of *Pseudalius tumidus*.† In stating that the form enclosed in cysts was inadequately described by Quekett (1841), Baylis & Daubney (1925) missed a later paper by him (Quekett, 1844) in which four pseudaliid species in *Phocoena phocoena* were described in some detail, and drawings of all four, including *Strongylus invaginatus*, were given. These drawings, though crude by modern standards, and the measurements given by Quekett, leave little doubt in my mind that *Strongylus invaginatus* Quekett, *Filaria inflexocaudata* v. Siebold, *Pseudalius tumidus* Schneider, and the forms described by later authors (Baylis & Daubney, 1925; Schuurmans Stekhoven, 1935;‡ Schmidt-Ries, 1939) under the name of *Halocercus inflexocaudatus* all belong to the same species. For this, as I have already pointed out (Dougherty, 1943*a*) in accordance with the

* For this record I am indebted to Dr V. B. Scheffer, of the Fish and Wildlife Division, U.S. Department of the Interior, Seattle, Washington, who sent me the preserved lungs of a female harbour porpoise.

† This name has been retained by Martini (1909) and by Chitwood & Chitwood (1937), who have reproduced Martini's figure of the distribution of nuclei in the lateral chords of this species.

‡ Schuurmans Stekhoven, by an obvious oversight, has unfortunately copied Schneider's figure of *Philometra* [*Ichthyonema*] *globiceps* as that of *Halocercus inflexocaudatus*.



Figs. 3–8. *Halocercus invaginatus*

Fig. 3. Posterior end of female, lateral view.

Fig. 4. Variation in position of vulva and anus relative to one another and to the posterior end of the female.

Fig. 5. Anterior end of female, lateral view.

Fig. 6. *En face* view of anterior end, dorsal side uppermost.

Fig. 7. Posterior end of male, ventrolateral view, spicules omitted.

Fig. 8. Posterior end of male, lateral view.

For explanation of lettering, see p. 82.

rule of priority, the correct name is *H. invaginatus*.*

The fullest existing account of this form is that of Schmidt-Ries (1939), who had an abundance of well-fixed specimens at his disposal and was able to describe it in detail. I am, however, able to extend Schmidt-Ries's description and in certain respects to correct it. That my observations should not in all cases agree with his may be readily appreciated by anyone who studies these delicate porpoise parasites, in which many structures are difficult to see at best and demand a certain unavoidable degree of subjectivity in interpretation. Schmidt-Ries's drawings of *H. invaginatus* are over-schematic and in some respects difficult to understand. But, by comparing them with his descriptions and with my material and by studying further detail in my own specimens, I have satisfied myself that we have dealt with the same species. I have had several dozens of both sexes of *H. invaginatus* for study.

The maximum dimensions of the specimens which I have studied are in general smaller than the measurements given by Baylis & Daubney and by Schmidt-Ries for their specimens. This same tendency has been shown in specimens of *Stenurus vagans* and of *Pharurus convolutus* which I have collected from the same hosts as specimens of *Halocercus invaginatus*. But size alone does not constitute a specific criterion, and otherwise these specimens seem morphologically typical of the species to which I have referred them. Maximum measurements given for *H. invaginatus* by Baylis & Daubney and by Schmidt-Ries are compared with mine in Table 2. Except for total body length these measurements were taken with a calibrated ocular micrometer. The arcuate spicules, the lengths of which are difficult to determine accurately on a linear basis, were measured along straight lines in three steps: first the capitulum (up to the slight constriction marking the calamus), then the lamina to the level of the beginning of the gubernaculum, and finally the remainder of the lamina.

The pattern of the cephalic papillae has already been discussed under the genus as a whole. For that of *H. invaginatus* see Fig. 6. The papillae were very difficult to see in my specimens. Only by study

Table 2. *Comparison of measurements in mm. for* Halocercus invaginatus *by Baylis & Daubney* (1925), *Schmidt-Ries* (1939), *and Dougherty* (*present work*).

	Baylis & Daubney	Schmidt-Ries	Dougherty
Maximum length: ♂	20	23	17
♀	—	65	31
Maximum width: ♂	0·25 (? with cuticle)	0·25 (? with cuticle)	0·17 (without cuticle)
♀	0·35 (? with cuticle)	0·4 (? with (cuticle)	0·17 (without cuticle)
Oesophagus: Length	0·195	—	0·165
Width	0·025	—	0·019
Spicules, length	0·2	—	0·205
Gubernaculum, length	0·055	—	0·045
Vagina vera + ovejector, length	0·35	—	0·225
Vulva to anus	0·062	—	0·063
Vulva to posterior end	0·1	—	0·088

of the same structures in *H. kirbyi* was I able to interpret the probable arrangement of these parts in *H. invaginatus*. The stoma and oesophagus in the latter species (Fig. 5) are typical of the genus.

In the male posterior end Schmidt-Ries illustrated the typical five bursal rays. These are figured here in Figs. 7 and 8. He spoke of two pairs of these, the ventral and 'dorsal' rays, as long in comparison with those of the previously known species of *Halocercus* and carrying two short papillae each. 'Dorsal' is obviously an unintended error for 'lateral'.* In my own material I have been able to make out three terminations for each lateral ray, two slender and digitiform, and one short and blunt; the first two of these are doubtless papillary, and the third may well be too, together possibly representing the three rays of the normal strongyline lateral ray-group. Schmidt-Ries spoke further of a pair of 'skin-flaps [Hautenlappen]' to the side of the ventral rays and often covering them. I see no reason for not considering each 'skin-flap' the base of a large and very thick pedunculate ventral ray, Schmidt-Ries's 'ventral ray' thus being the termination of the ray. This was apparently the interpretation by Baylis & Daubney for *H. delphini*, in which the termination was reported to be sessile, and for *H. lagenorhynchi*, in which the termination was reported to be pedunculate. I have not been able to make out two terminations, which Schmidt-

* *Strongylus vagans* Eschricht is not, as was tentatively suggested by Baylis & Daubney (1925), a synonym of *H. invaginatus*, but should now be known as *Stenurus vagans* (Eschricht, 1841) Dougherty, 1943 (syn. *Stenurus minor* (Kuhn, 1829) Baylis & Daubney, 1925). Contrary to statements by Baylis & Daubney, v. Siebold (1842) did not attribute the name *Strongylus inflexus pulmonalis* to Eschricht, nor did Eschricht (1841 *c*, *d*) record *Strongylus vagans*. In earlier papers on the lungworms of the harbour porpoise Eschricht (1840, 1841 *a*, *b*) made no reference to forms in nodules, but in his later works these are mentioned as the young of *Strongylus* [*Pseudalius*] *inflexus*.

* 'Die ventralen (*vR′* und *vR″*) und die *dorsalen* (*dR′* und *dR″*) Rippen sind im Vergleich zu denen der bisher bekannten...Arten lang und tragen am Ende zwei kurze Papillen. Die *dorsale* Rippen (*mR*) ist stark reduziert' [italics mine]. Since there are no designations *dR′* and *dR″* in Schmidt-Ries's figures (Abb. 21 and 22), but *lR′* and *lR″* instead, 'dorsalen (*dR′* und *dR″*)' should doubtless read 'lateralen (*lR′* und *lR″*)'.

Ries indicated in the text of his paper, but not in his drawing of the male bursa. In agreement with his observation the bursa of *H. invaginatus* seems undivided. The spicules are much shorter than those in the genotype, being simply arcuate with inconspicuous alae and distinct sheaths. The gubernaculum is short, only slightly curved, and rather weakly sclerotized.

From Schmidt-Ries's diagram of the female posterior end in lateral view (Abb. 24) and my own drawing (Figs. 1A, 3) it would appear that the difference between the two is so great that they cannot represent the same species. However, in Fig. 4 I have presented a series showing the considerable variation to be encountered in the relative proportion of the distances between anus and vulva and between both and the posterior tip of the body, ranging from the first one in the series, which is nearest to the figure in Schmidt-Ries's paper, although not so extremely foreshortened, to the last, which represents the same specimen as in Fig. 3. Baylis & Daubney spoke of the tail of the female as being relatively more elongate than that of the other species of the genus; hence they doubtless dealt with specimens at the latter end of the series. Quekett's picture (1844) of this part also shows a long tail. Such forms in my own material were more numerous, hence more typical.

Schmidt-Ries interpreted the posterior end of the female pseudaliid as consisting fundamentally of three 'rays' as in the male—a single dorsal and paired lateral rays; these differed from the same rays in the male, however, in being rather closely bound together, thus maintaining the typical rounded outline of the female tail. Such an interpretation deserves analysis. If a female strongyline nematode possesses a tripartite posterior end, the three elements cannot be considered homologous to both dorsal and *lateral* rays of the bursa of the male, inasmuch as the female posterior end as a whole is homologous to the male dorsal ray alone, as Looss (1905) first showed for *Ancylostoma* Dubini, 1843, *emend.* It is thus desirable to determine the nature of the structure described by Schmidt-Ries. I have not been able to discern the tripartite division of the female posterior end in most of my specimens of the two species of *Halocercus* studied, nor of species in other genera of pseudaliid nematodes, but in a few I have been able by varying the focus of the microscope to achieve an effect resembling Schmidt-Ries's figures. Possibly this difference in observation may be explained on the basis of different fixation methods employed by Schmidt-Ries and myself. He further observed what he called 'spines [Zipfeln]' on the 'lateral rays' of the female. It is apparent both from his drawings and from my own observations that his 'spines' are actually the phasmids. The 'lateral rays' themselves were apparently in part at least the outlines of the phasmidial glands, although this matter can best be conclusively determined by sectioning methods. Thus Schmidt-Ries's interpretation of the female posterior end in the Pseudaliidae—resulting fundamentally from ignorance of certain details of nematode comparative anatomy—is untenable.

The vulva of *H. invaginatus* is characterized by two lips, which with a small amount of adjacent tissue probably represent the vagina vera. The reservoir and the terminal parts of the paired uteri are filled with larvae, and successive stages are to be found from the germinal end of the ovary through egg-production, cleavage stages, and embryogenesis, to the unlaid embryo, which escapes from the female free of a shell.

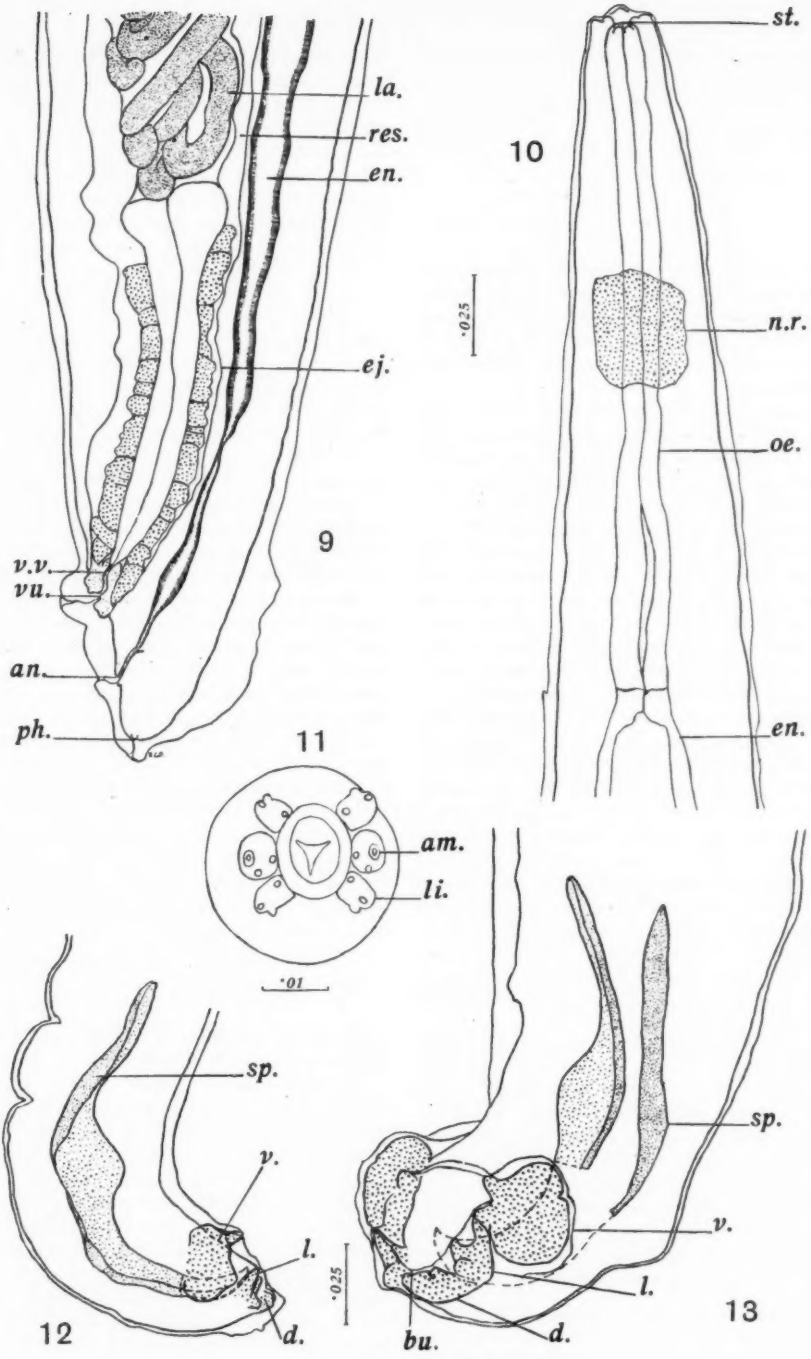
Halocercus kirbyi n.sp.

(Figs. 9–13)

Synonymy: *Halocercus* sp., Benson & Groody, 1942, p. 44. *Halocercus* sp., Dougherty, 1943*a*, pp. 19, 21.

Diagnosis. Length, male 30–40 mm., female 52–88 mm. Maximum thickness (including cuticle), male 0·125 mm., female 0·150 mm. Cuticle only slightly irregular, but tending toward a somewhat swollen condition in the female posterior end; general thickness about 0·01 mm. Male posterior end bent ventrally. Oral opening equipped with six well-developed lips. Oesophagus short; length, male and female 0·17–0·21 mm.; maximum width, male 0·017–0·021 mm., female 0·016–0·021 mm. Nerve ring a little over one-third length of oesophagus from anterior end. Excretory pore not observed, but subventral glands can be followed with difficulty and their terminations and giant nuclei made out. Male genital system: bursa just barely evident; ventral ray with one pedunculate termination on a relatively very large base, lateral ray with three terminations, one short and blunt, two longer, more or less pointed, and subequal, dorsal ray with two blunt terminations; spicules very short, 0·125–0·145 mm. long, arcuate, trough-like, with an abrupt expansion of the alae on both sides of the middle of each spicule; gubernaculum not observed; testis extends forward to within about 3 mm. from anterior end. Female genital system, vulva with distinct lips, 0·021–0·038 mm. from anus and 0·036–0·069 mm. from posterior end; combined length of vagina vera and ovejector 0·305–0·615 mm.; vagina vera, about 0·010 mm. long; ejector, 0·090–0·180 mm. long; reservoir filled with a single line of embryos; anterior ovary extends forward to as much as one-eighth of body length from anterior end.

Holotype male and allotype female: in the United States National Museum (U.S. Nat. Mus. Helm. Coll. Nos. 36867 and 36868 respectively); paratype male and female in the British Museum (Natural History).



Figs. 9–13. *Halocercus kirbyi*

Fig. 9. Posterior end of female, lateral view.
Fig. 10. Anterior end of female.
Fig. 11. *En face* view of anterior end.
Fig. 12. Posterior end of male, lateral view.
Fig. 13. Posterior end of male, ventrolateral view.
For explanation of lettering, see p. 82.

Type and only host. *Phocoenoides dalli* (True), Dall porpoise.

Habitat. Nodules or small cavities near the surface of the lungs.

Geographic distribution. Type locality: San Francisco Bay, California.

Discussion

Because of difficulty in extracting specimens of *Halocercus kirbyi* from nodules which they occupied near the surface of their host's lungs only four male and four female specimens were recovered entire, in addition to numerous anterior and posterior ends of both sexes.

H. kirbyi is apparently closest to *H. invaginatus* in the ventral flexion of the male posterior end, general bursal structure, and length of the spicules. Other apparent similarities rendering these two species more closely related to one another than to other species of the genus may actually be due to the fact that both *H. kirbyi* and *H. invaginatus* have been studied in some detail and comparatively, and equivalent observations on several characters are lacking for other species. *H. kirbyi* differs from *H. invaginatus* in the shape and nature of the spicules, which are unlike those of any other species (Figs. 12, 13); in the consistently longer ejector of the ovejector (Fig. 9); and in the much more strongly developed lips about the oral opening (Fig. 11). *H. kirbyi* has the shortest spicules of any species so far described in *Halocercus*. Its bursal rays appear to be relatively the stoutest. The stoma and oesophagus (Fig. 10) are characteristic of the genus.

I have studied the male posterior end of *H. kirbyi* in some detail by means of the oil-immersion lens. It has seemed possible that additional tiny papillae may exist in the posterior region of the genital cone, but my material was too limited and my observations too subjective to permit a definite conclusion. It is likely that more refined techniques, such as sectioning, are desirable to elucidate this and other points in the morphology of the delicate and degenerate pseudaliids of porpoises.

SUMMARY

The present paper includes two sections of a proposed series on the lungworms of the Odontoceti or toothed whales. Only members of the family Delphinidae among the Odontoceti have so far been reported as hosts to these lungworms.

The first section deals with the systematic relationships of the lungworms of the Odontoceti. They are held to constitute a family Pseudaliidae in the superfamily Metastrongyloidea. The nature of the female reproductive system and specifically of the ovejectoral apparatus in the suborder Strongylina is discussed and the concept expressed that the organization of the latter structure is of phylogenetic and taxonomic importance in this group. On the basis of variations in the ovejectoral apparatus three families of strongyline lungworms (except for *Syngamus* v. Siebold, 1836) are recognized —Dictyocaulidae Skriabin, 1941, Metastrongylidae Leiper, 1909, and Pseudaliidae Railliet, 1916.

The second section deals with the genus *Halocercus* Baylis & Daubney, 1925. Previous literature is reviewed. A key and table of measurements for the known species are presented. *H. invaginatus* (Quekett, 1841) Dougherty, 1943, is redescribed, and *H. kirbyi* n.sp. named and described.

ADDENDUM

Since the manuscript of this work was submitted for publication, Dr H. A. Baylis, of the British Museum (Natural History), has very kindly drawn my attention to a paper by Skriabin (1942)* in which the lungworms of porpoises were discussed in some detail. This work included diagnoses of the two genera *Skrjabinalius* Deliamur in Skriabin, 1942, and *Otophocaenurus* [sic] Skriabin, 1942, which Skriabin had already mentioned in 1941. On the basis of his earlier paper I listed them (Dougherty, 1943*a*) as unconfirmed genera, and in the first paragraph of the introduction to this paper I have again referred to them as unverified.

Skriabin's second paper directly concerns the subject-matter of the present work, as a new species of *Halocercus*—*H. tauricus** Deliamur in Skriabin, 1942—was mentioned, and he proposed to split the genus into three subgenera: *Halocercus*, *Prohalocercus* Skriabin, 1942, and *Posthalocercus* Deliamur in Skriabin, 1942. Also, I consider the genus *Skrjabinalius* to be synonymous with *Halocercus*.

Although *Halocercus tauricus* was figured and certain distinguishing characters discussed, which allow me to accept it as a distinct species, no diagnosis was presented, and, since the nature of its spicules

* The original (in Russian) of this paper, in *Doklady Akad. nauk S.S.S.R.* **37**, suffers considerably by a poor translation (into English), in *C.R.* (*Doklady*) *Acad. Sci. U.R.S.S.* **37**, 35–40, the foreign language edition of the article.

* This was given by Skriabin as *H. taurica*, but I emend the specific name to *tauricus* here.

is unknown to me, I cannot indicate where it would fall in the key included in this paper. Its type and only host is the Black Sea harbour porpoise (*Phocoena relicta*).*

I do not subscribe in general to the use of subgenera in nematode genera with few species, and in the case of *Halocercus* there is basis for rejecting Skriabin's subgenera further than a mere disapproval on principle. These subgenera are related to his concepts of pseudaliid evolution. In the first section of this paper I avoided any discussion of Skriabin's phylogenetic theories. I intend to criticize them at length in a later work in which direct evidence to substantiate my views will be presented. However, inasmuch as Skriabin regards the genus *Halocercus* as having considerable evolutionary importance, a few words on his ideas are necessary here. He believes, as expressed in both papers, that the lungworms of porpoises are not degenerate forms, but primitive strongylines in which the bursa and its rays have never developed to the degree found in strongyloids, trichostrongyloids, and most metastrongyloids; *Halocercus*, in which the bursa and rays are least well developed, is the most primitive genus. The subgenus *Prohalocercus*, with two species, *Halocercus* (*Pro.*) *inflexocaudatus* (= H. *invaginatus*) and *H.* (*Pro.*) *gymnurus* (= *Filaroides gymnurus*), was supposedly characterized by complete lack of a bursa. This is true of the bursa, but not of the bursal rays of *H. invaginatus*, and, although probably accurate for *Filaroides gymnurus*, the relationships of this form, as I have indicated elsewhere (Dougherty, 1943*c*), are with the genus *Aelurostrongylus* Cameron, 1927, and other metastrongylid genera in carnivores. The subgenus *Halocercus* for the rest of the species of *Halocercus*, except *H. tauricus*, was characterized by a rudimentary, unlobed bursa and only slightly developed rays. The subgenus *Posthalocercus*, for *Halocercus* (*Post.*) *taurica* (= *H. tauricus*), was distinguished by a lobulated bursa and rather clearly subdivided rays. In my opinion the supposed distinctions between these subgenera are of little consequence, for I believe that a restudy of the species of which I have had no material will show that the condition of the rays in all is essentially as I have shown for *H. invaginatus* and *H. kirbyi*, save that in all probability the double termination recorded by Lins de Almeida (1933 *a*, *b*) for the ventral ray of *H. brasiliensis* sets this species aside from all except *H. tauricus*—figured by Skriabin as also having a doubly terminated ventral ray.

The series *Prohalocercus-Halocercus-Posthalocercus* is used by Skriabin as an evolutionary line of increasing bursal complexity, leading toward the other lungworm genera in porpoises and through them to the typical metastrongyloids, which in turn have given rise to the superfamily Strongyloidea. To me the use of *Halocercus* as a stem form implies a complete reversal of logic. I cannot agree that the pseudaliids, inhabitants of an aberrant mammalian group, could ever have given rise to other strongyline groups. It is necessary either to conceive of them as spreading from these hosts or their immediate ancestors to all vertebrate classes above the fishes or to regard them as the last survivors of a once more widely spread assemblage. Either theory is far less tenable than that the Pseudaliidae represent the end-product of a degenerative evolution from a more elaborate strongyline type. Furthermore, Skriabin's concept that the Strongyloidea arose from the Metastrongyloidea is contradicted by embryological evidence; in every respect the strongyloids are closer to the Rhabditina than are the metastrongyloids. The strongyloid first-stage larva has a typical rhabditoid stoma and oesophagus, whereas the metastrongyloid first-stage larva shows only a suggestion of rhabditoid structure; obviously the latter are more specialized forms derived from other strongylines, probably the strongyloids, and not directly from free-living nemas.

I regard *Skrjabinalius* Deliamur in Skriabin, 1942, as insufficiently different from *Halocercus* to justify its maintenance as a separate genus. Indeed, I suspect that *H. cryptocephalus* (Deliamur in Skriabin, 1942) n.comb. (syn. *Skrjabinalius cryptocephalus*) may actually be the same as *H. delphini*. These species both occur in the same species of host, *Delphinus delphis* Linné,* and their measurements agree except for the length of the oesophagus, which as given for *Halocercus delphini* is almost twice that of *H. cryptocephalus*. However, I have found specimens of *H. invaginatus* with oesophagi as short as 0·105 mm. as compared with 0·200 mm. indicated by Balis and Daubney. It is true that the rays as figured by Skriabin for *H. cryptocephalus* show greater subdivision that those figured by Baylis & Daubney for *H. delphini*; however, a restudy of *Pharurus convolutus* (Kuhn, 1829) Dougherty, 1943, and *Stenurus vagans* (Eschricht, 1842) Dougherty, 1943, both figured by Baylis & Daubney, has divulged a correspondingly more complex structure of the rays than shown for these species by the latter authors. Nevertheless, in lack of more direct evidence it seems advisable to leave *Halocercus cryptocephalus* as an independent species pending further investigation. Its position in the key cannot be stated because of the absence of figures of the spicules.

* Zalkin (1938, *Zool. Zh.* **17**, 706–33) considered this form a subspecies of *Phocoena phocoena*, viz. *P. p. relicta* (Abels). His work is thorough and in all probability reliable.

* *H. cryptocephalus* occurs in the Black Sea subspecies of the common porpoise, viz. *Delphinus delphis ponticus* Barabash-Nikiforov.

Skriabin proposed to subdivide the family Pseudaliidae into four subfamilies: Filaroidinae Skriabin, 1933; Skrjabingylinae Skriabin, 1933 (with *Skrjabinalius* and *Skrjabingylus*); Pseudaliinae Railliet and Henry, 1909 (with *Pseudalius*); and Stenurinae Skriabin, 1942 (with *Stenurus*, *Torynurus* [=*Pharurus*], and *Otophocaenurus*). A fifth subfamily, Propseudaliinae Skriabin, 1942, was postulated as a hypothetical group from which the Filaroidinae and *Halocercus* arose. *Halocercus* was not included in any subfamily and was regarded as the stem genus from which Skrjabingylinae, Pseudaliinae, and Stenurinae arose. A hypothetical genus *Prostenurus* Skriabin, 1942, was suggested as intermediate between *Halocercus* and the Stenurinae. Without the context of Skriabin's phylogenetic ideas this classification loses meaning. The subfamily Filaroidinae belongs in the family Metastrongylidae and has no direct connexion with *Halocercus*. *Skrjabingylus* Petrov, 1927, which Skriabin believes has arisen from *Skrjabinalius*, is a dictyocaulid genus and has no direct connexion with the Pseudaliidae. There seems no advantage in dividing the family Pseudaliidae *sensu stricto* into subfamilies Pseudaliinae and Stenurinae.

The status of *Otophocaenurus* Skriabin, 1942, will be discussed in a later section.

Skrjabinalius Deliamur in Skriabin, 1941, and *Otophocoenurus* [*sic*] Skriabin, 1941, must be regarded as *nomina nuda*. The names themselves, however, have been established in Skriabin's later work (1942), the latter as *Otophocaenurus* [*sic*].

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MORE CASES OF ABNORMAL DEVELOPMENT IN THE ARGASID TICK, *ORNITHODORUS MOUBATA* MURRAY

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(With 2 Figures in the Text)

1. ABNORMALITIES OF THE LEG

During the handling of about 10,000 ticks in the course of insecticidal tests many abnormalities of the legs were encountered. Most of the cases were deficiencies due to the partial regeneration of a leg damaged in the previous nymphal stage. Two cases of special interest were not deficiencies, but exhibited supernumerary segments of leg.

The first case (Fig. 1A) concerns the second right leg of a third-stage nymph. The tibia bore two extra tarsi and protarsi which were slightly less than normal size and formed perfectly except that the tarsal humps were not prominent. The two segments were joined proximally and suspended by a slender ligament from the main limb.

The second abnormality occurred in a fourth-stage nymph which moulted to become a male. The abnormal leg was the third left and it appeared unchanged after the moult. Here the base of the tarsus had a lateral projection bearing two supernumerary tarsi of normal size (Fig. 1B). The tarsal humps were fully formed.

Species	Reported by	Segment bearing supernumerary members
Hyalomma aegyptium ♂	Nuttall (1914)	Trochanter (2nd right)
Amblyomma dissimile ♂	Brumpt (1934*a*)	Femur (2nd right)
Dermacentor niveus ♀	Olenev (1931)	Femur (4th left)
Ornithodorus moubata nymph	Present author	Tibia (2nd right)
Dermacentor niveus ♀	Olenev (1931)	Protarsus (4th left)
Ornithodorus moubata ♂	Present author	Base of tarsus (3rd left)
Hyalomma aegyptium ♂	Sharif (1930)	Extremity of tarsus (4th left)

Abnormalities of the leg have been noticed before in ticks and have been reported in *Hyalomma aegyptium* by Nuttall (1914) and Sharif (1930); in *Dermacentor niveus* by Olenev (1931) and in *Amblyomma dissimile* by Brumpt (1934*a*). It is interesting to note that the branching of the limb may take place at all levels as shown in the above table.

These reduplications often occur as a result of regeneration after injury (Wigglesworth, 1939).

2. PARTIAL TWINNING

This condition has been noticed before in *Ornithodorus moubata* (Robinson, 1943) when duplication of anus, anal muscles and marginal muscles was reported in two individuals. The present specimen was a female (Fig. 2) first noticed as a second-stage nymph, and in it the twinning was much more complete. The whole of the region behind and including the spiracles was duplicated, and in addition a central area in front of the spiracles including the vulva. The twinned vulvae were slightly smaller than usual and inclined to one another at an acute angle (Fig. 2, *l.v.*). The apposed spiracles (*ap.sp.*, named 'posterior' by Brumpt), were not quite separated, and in front of them was a deep pit in the cuticle.

The tick mated normally, the spermatophore being applied to her right-hand vulva. She was given several chances of feeding over a period of a month from the last moult, but she refused. Nevertheless, she laid a small batch of eggs, which is not particularly unusual for an unfed female (Robinson, 1942), and a few developed into normal ticks. Sterility of a number of them was probably caused by the vulva being at an oblique angle to the long axis of the body. Because of this the eggs were not all delivered into the arms of the organ of Géné, and therefore did not all develop successfully.

In the literature on ticks various degrees of twinning are reported, and the following list summarizes the external characters of the types with two anal apertures:

(1) Posterior spiracle absent: *Boophilus decoloratus* ♂ (Warburton & Nuttall, 1909), *Rhipicephalus appendiculatus* ♂ (Nuttall, 1914), *Hyalomma aegyptium* ♀ (Senevet, 1922), *Amblyomma dissimile* ♂ (Brumpt, 1934*b*), *Ornithodorus moubata* ♂ and ♀ (Robinson, 1942).

(2) Posterior spiracle single: *Rhipicephalus san-*

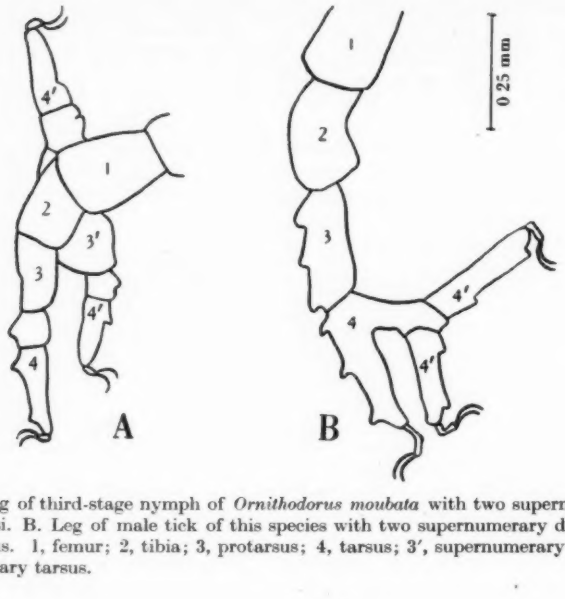

Fig. 1. A. Leg of third-stage nymph of *Ornithodorus moubata* with two supernumerary tarsi and protarsi. B. Leg of male tick of this species with two supernumerary distal segments of the tarsus. 1, femur; 2, tibia; 3, protarsus; 4, tarsus; 3′, supernumerary protarsus; 4′, supernumerary tarsus.

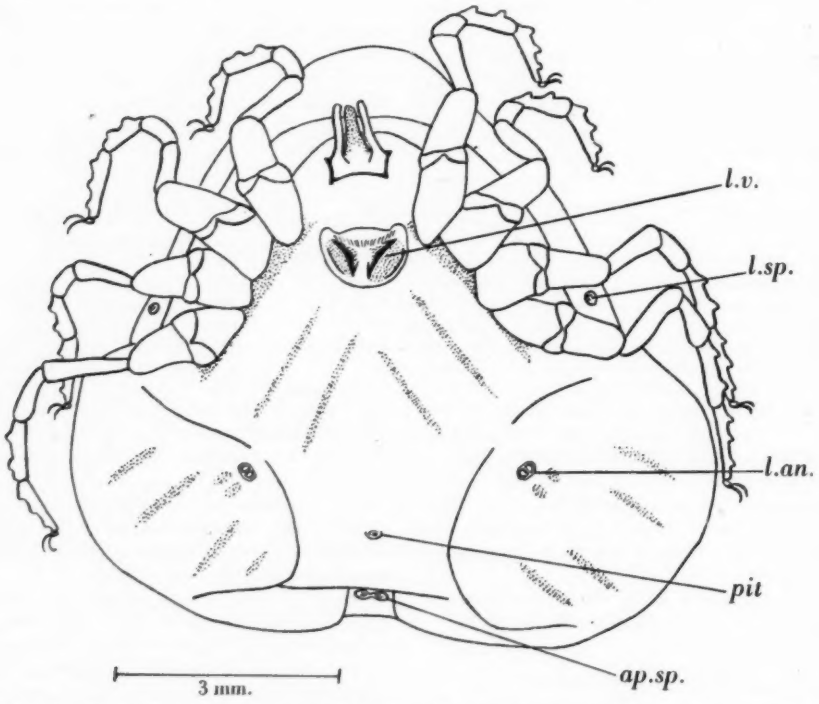

Fig. 2. Ventral view of the partially twinned ♀ *Ornithodorus moubata*. *ap.sp.* apposed spiracles; *l.an.* left anus; *l.sp.* left spiracle; *l.v.* left vulva.

guineus ♂ (Warburton & Nuttall, 1909), *Ixodes hexagonus* (Brumpt, 1934*b*).

(3) Posterior spiracles two and opposed: *Ixodes hexagonus* nymph, *Rhipicephalus bursa*, nymph (Brumpt, 1934*b*), *Ornithodorus moubata* ♀ (present paper).

(4) Posterior spiracles two and widely separated, fourth pair of legs twinned: *Ixodes ricinus*, nymph (Brumpt, 1934*b*).

The internal organs behind the genital opening were completely duplicated, the two sets being separate. The two alimentary canals merged into each other at the level of the oesophagus.

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STUDIES ON THE ANTHELMINTIC ACTIVITY OF HEXYLRESORCINOL AND TETRACHLORETHYLENE

By W. P. ROGERS, *from the Molteno Institute, University of Cambridge*

(With 9 Figures in the Text)

INTRODUCTION

Laboratory studies on anthelmintics may be undertaken by *in vitro* methods or by *in vivo* techniques using small animals. The practical importance of results obtained by such methods is limited if anthelmintics are specific in action. However, though it appears from the literature (for references see Findlay, 1939) that a low-grade specificity may exist, this may be determined by the physical and chemical nature of the parasites' environments rather than by the parasites themselves. It therefore seems reasonable to study anthelmintics along three lines: (*a*) *in vitro* tests on the toxicity of drugs to parasites, (*b*) tests on drug toxicity to host animals, and (*c*) the examination of the effects of the physical and chemical properties of the parasites' normal environments on drug activity. Having found the conditions for maximal anthelmintic activity, a drug which shows a relatively low toxicity to host animals should then be examined by *in vivo* tests.

In vitro tests may be undertaken by the Lamson & Brown technique (1936) or better by Baldwin's (1943) modification of the Trendelenburg (1916) kymographic method. The effects of the physical and chemical properties of the parasites' normal environments on drug activity had received little attention until 1943, when Trim studied the action of bile salts and mucin on the rate of penetration of hexylresorcinol into *Ascaris lumbricoides*. The present work has been undertaken in an attempt to supplement these *in vitro* tests and to develop a laboratory method for checking by *in vivo* tests the results so obtained. Since, as the result of Trim's (1943) work, the mode of action of hexylresorcinol is better known than that of most other anthelmintics, this drug was selected for testing techniques. Tetrachlorethylene was also examined to determine, if possible, those properties by virtue of which it exercises anthelmintic activity against a wide variety of parasites.

IN VITRO TESTS

In selecting the *in vitro* test to be used the following points were considered: (1) the parasites should be examined in an environment similar in its chemical and physical characteristics to the natural habitat, and the various elements of the environment should be open to some degree of control; (2) changes of 20 % in drug activity should be detectable; (3) some indications as to the pharmacological properties of the drug should be obtainable. The method which satisfies these conditions consists in perfusing the drugs through the lumen of a section of rat intestine infected with *Nippostrongylus muris*. This parasite was chosen as a test organism because it is easily maintained in the laboratory, it bears a close taxonomic relationship to the economically important Trichostrongyle parasites of sheep, and it lives in an environment similar to that of the Trichostrongyles most refractory to treatment.

The preparation of test materials

Minor variations in the condition of the test materials, such as the presence or absence of food or changes in bile-salt concentration, affected the results obtained, and careful standardization of technique was necessary. Black-hooded rats, 8–10 weeks old, fed for 2 weeks before infection on 'rat cake' (Thomson, 1936), milk and green vegetables, were infected by subcutaneous injection of about 1500 larvae obtained from faeces cultured on damp charcoal. Before use, the animals were starved overnight in cages equipped with coarse wire grills which allowed the faeces to fall out of reach of the rats. 11–15 days after infection as required, rats were killed with ether, the first half of the small intestine immediately removed, and a heavily infected section, 12 cm. long, selected and attached to the perfusion apparatus. Infected regions of the intestine could be detected by the local distension and red colour caused by the 'worm nests'.

The method of perfusion

Fig. 1 shows the apparatus used. The infected intestine was held by rubber bands on to the cannulae *a* and *b* and immersed in a bath containing 500 ml. of aerated Tyrode-glucose solution at 38°C. Perfusion through the lumen of the intestine was then commenced by allowing the drug, suspended or dissolved in a suitable medium, to pass through the apparatus at a pressure of 3 cm. of water to reach a position of equilibrium as shown. For the injection of drug into the lumen of the intestine

during the course of the experiment, the intestine was perfused with saline from a bulk container and the drug injected from the syringe *d* as required. The escape of the drug before the plunger of the syringe was pressed was prevented by drawing a little air into the needle. If the action of the drug on intestinal movements was being examined, the bulk container was stoppered and a sensitive volume

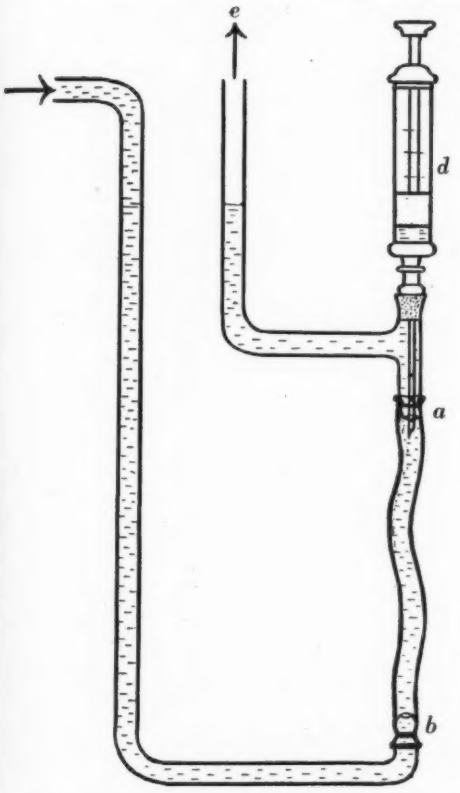

Fig. 1. Showing the apparatus used for perfusing the lumen of the infected intestine. For explanation see p. 98.

tambour connected at *e* after the perfusion solution had reached a position of equilibrium. Volume changes were then recorded kymographically.

The estimation of parasite mortality

After perfusion for a given time, the section of gut was removed from the cannulae, placed in 200 ml. of aerated Tyrode-glucose solution at 38° C. and the parasites freed in the solution by scraping the mucosa with a blunt scalpel. The worms were then collected in a pipette and transferred to another 200 ml. of the solution saturated with a 95 % O_2, 5 % CO_2 gas mixture at 40° C. contained in a counting dish which had a white base marked off in numbered 1 cm. squares. After ½ min. the proportion of active worms was determined with a hand lens over a period of 4 min. The parasites were examined in the same manner after being incubated in saline for 1 hr. The accuracy of thus determining the proportion of inactive worms was examined by killing known numbers of parasites with heat or 1/1000 CCl_4 in 0·9 % saline and then mixing them with known numbers of worms which had been incubated in saline for 2 hr. Table 1 summarizes the results obtained. The relationship between the proportion of inactive worms and drug efficiency is discussed later (see p. 106).

Table 1. *Showing the proportions of active parasites observed in known mixtures of dead and alive worms*

Percentage of active parasites		No. of parasites examined
Calc.	Obs.	
58	52	92
54	60	129
40	41	98
57	48	148
37	40	166
53	46	115
45	40	128
98	96	82
10	14	108
72	65	112

The effects of mucus on drug efficiency

Trim (1943) has shown that gastric mucin inhibits the action of hexylresorcinol. As it seemed that mucin might also form a physical barrier to drugs, the ability of anthelmintics to attack parasites through mucin was investigated by determining the proportion of worms inactivated by drugs free in the intestinal lumen and under the mucus. Thus, when worms were examined after perfusion, those free in the lumen and those which had to be scraped from the mucosa were studied separately.

If mucin does protect parasites, irritant anthelmintics may have their efficiency reduced by mucus secreted in response to the irritation. The effect of hexylresorcinol and tetrachlorethylene on mucin secretion or retention was therefore examined by estimating the amounts of mucus per unit weight and unit area of gut before and after perfusion. To determine the amount present before perfusion, a section of intestine about 1·5 cm. long, free from parasites and adjacent to the part selected for perfusion, was placed in warm saline containing 1/100,000 adrenaline-atropine for 15 min. This sample was then opened, trimmed with a scalpel,

measured and the mucus scraped free. Mucus and gut wall were then dried at 100° C., weighed and the mucus concentration calculated. A similar procedure was used to determine the mucin present after perfusion. To obtain a satisfactory sample for this purpose, it was necessary, when selecting the 12 cm. of gut for perfusion, to take a portion containing a short region free of worms to be used for the mucin estimation because, apart from the difficulty of freeing the mucus from parasites, regions where 'worm nests' were present appeared distended, and it seemed probable that the worms themselves may have caused localized increased mucin secretion or a thickening in the gut wall.

The normal variation in the amounts of mucus found along the intestines of rats was examined in the above manner (see Table 2). Rat 1 (starved overnight) provided samples taken at 6 cm. intervals along the gut and rat 2 (intestine contained food) adjacent samples, 2 cm. long.

Table 2. *Showing the amounts of mucus found on samples 6 cm. apart (rat 1) and on adjacent 2 cm. sections of gut (rat 2)*

Mg. mucus per cm.² gut wall		Mg. mucus per 10 mg. gut wall	
Rat 1	Rat 2	Rat 1	Rat 2
4·9	9·3	4·1	7·8
6·2	12·1	5·3	10·7
7·3	11·4	4·7	9·9
5·9	10·4	3·4	9·3
6·0	8·7	5·3	8·3
9·0	11·3	6·3	7·9

Perfusion time

Rat intestine, perfused through the lumen with 0·9% saline buffered to *p*H 6·5, remained active and, except for a slight fall in tone, seemed fairly normal for at least 4 hr. It is doubtful, of course, if the mucosa would be normal after such a time. However, the relationship between time and the number of worms inactivated by 1/2000 hexylresorcinol in buffered saline containing 1/2000 sodium oleate was examined at intervals up to 4 hr. (see Fig. 2). Tetrachlorethylene, 1/4000, was examined similarly (see Fig. 3). Evidently perfusion time up to 2 hr. was important. As it seemed possible that drugs would not be in contact with parasites in the small intestine of the rat for as long as 2 hr., the movement of the water-barium meal was studied to obtain indications of what might be a reasonable perfusion time.

Rats (starved overnight) were lightly anaesthetized with ether and 1·5 ml. of $BaSO_4$ emulsion delivered directly into the stomach by means of a soft rubber catheter. Immediately after dosing, and at frequent intervals up to several hours, the position of the barium meal was determined by X-ray photography. Under these conditions the stomach emptied rapidly and the $BaSO_4$ traversed the first half of the small intestine in about 90–120 min. The four rats examined showed the barium meal, 30, 45 and 60 min. after dosing, occupying positions in the region where *N. muris* is normally found in rats 8–10 weeks old. An hour later, the barium had passed this region in all the rats. It appeared that 1 hr. would be a reasonable time for the perfusion, but experiments to determine the rate of passage of hexylresorcinol along the rat alimentary canal showed that the drug left the stomach slowly and the concentration reached in the intestine was low but was maintained for at least several hours. This was probably partly due to the effect of the drug on

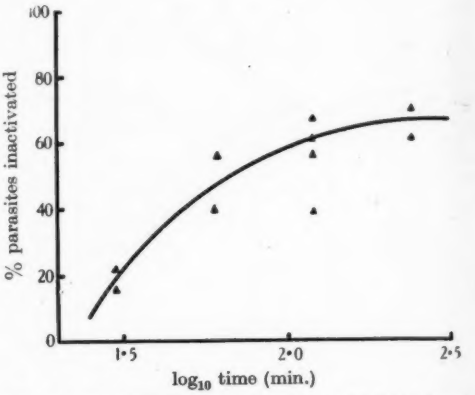

Fig. 2. Showing the rate of action of 1/2000 hexylresorcinol in buffered saline containing 1/2000 sodium oleate.

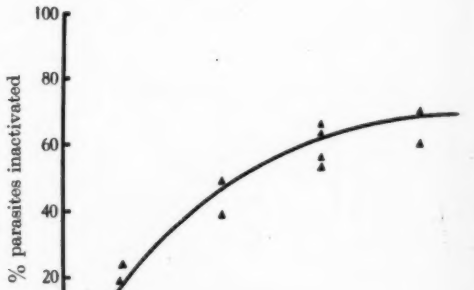

Fig. 3. Showing the rate of action of 1/4000 tetrachlorethylene in buffered saline.

peristalsis, for it was found that the activity and tone of the hexylresorcinol perfused gut soon fell off, and partly to the tendency of the drug to become adsorbed on the mucin of the stomach and intestine. In examining the anthelmintic activity of hexylresorcinol, therefore, a perfusion time of 2 hr. was adopted. Though C_2Cl_4 probably passed along the intestine more rapidly a similar perfusion time was used for purposes of comparison.

The dosage-mortality curve

The perfusion fluid used consisted of varying amounts of anthelmintic in 0·9 % saline containing 10 % of its volume of isotonic phosphate buffer at *p*H 6·5. Hexylresorcinol was examined in the presence of 0·2 % sodium oleate to allow higher concentrations to be used, and the hydrogen-ion concentration was selected as being about that of the region of the rat gut where *N. muris* was normally found (Watchorn, 1932). Perfusion fluids without anthelmintics had little action, inactivating less than 2 % of the worms (four tests). 'Dosage-mortality' curves are shown in Figs. 4*B* and 5.

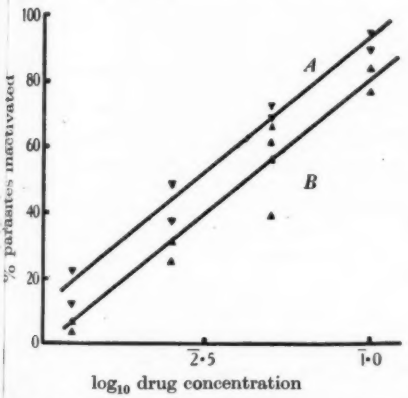

Fig. 4. Showing the inactivation of parasites in *A*, buffered saline containing *M*/3000 ascorbic acid, 1/2000 sodium oleate and varying amounts of hexylresorcinol and *B*, as in *A* but without ascorbic acid. Hexylresorcinol has been plotted as the logarithm of the percentage concentration.

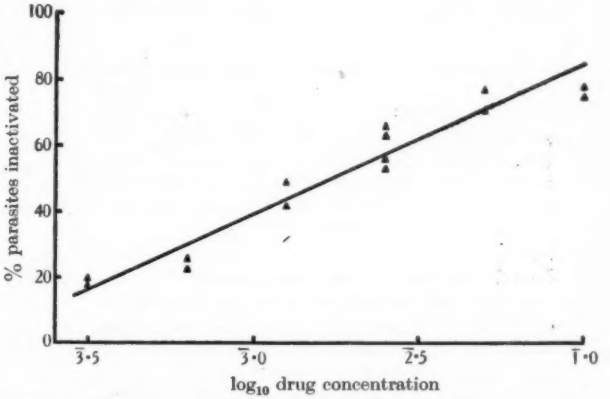

Fig. 5. Showing the inactivation of parasites by varying amounts of tetrachlorethylene in buffered saline. Drug concentration is given as the logarithm of the percentage concentration.

Although the conditions of these experiments were such as to loosen the mucus surrounding the worms and hence drug penetration was much better than could be expected under natural conditions, the proportion of parasites affected by the anthelmintics was always greater among worms found free in the intestinal lumen than among worms adhering to the mucosa. Thus the ratio

$$\frac{\%\text{ inactivated under mucus}}{\%\text{ inactivated in gut lumen}}$$

varied from one-third to one-sixth at low concentrations of C_2Cl_4 and from three-quarters to one-half at high concentrations. The different dosage effects were even more marked than these figures indicate, for the proportion of parasites found in the mucus was greater when low drug concentrations were used. Somewhat similar results were obtained when hexylresorcinol was used, though relatively more worms were usually found under the mucus in this case.

The effects of detergents on drug activity

The action of sodium oleate was studied by perfusing the lumen of the gut with varying amounts of soap in 0·9 % saline solution buffered at *p*H 6·5 and containing 1/2000 hexylresorcinol. The results of these experiments summarized in Fig. 6 show that the hexylresorcinol was most active in the presence of 0·2 % sodium oleate. The soap itself, in the absence of an anthelmintic, showed a rapidly increasing activity when the concentration rose above 0·2 %. This effect was not necessarily due to the high osmotic pressure, for perfusion fluids containing 2 % bile salt were not toxic to *N. muris*, and further, sodium laurate gave a similar dosage-mortality curve (Fig. 7) at much lower concentrations.

Since the increase in anthelmintic efficiency caused by sodium oleate was probably in the nature of a surface activation, sodium laurate, which is much more active at *p*H 6·5 (Jarisch, 1922) was also examined. In this case 100 % of the parasites were inactivated by 1/2000 hexylresorcinol in the presence of 0·125 % soap though more than 90 % were similarly affected when the laurate concentration reached 0·031 % (Fig. 6). This rapid rise in activity

was largely due to the soap alone, for concentrations of 0·5 % killed almost all the worms. The effects of sodium laurate on the parasites was most marked, inactive worms being almost colourless and the tissues frequently appearing to be macerated.

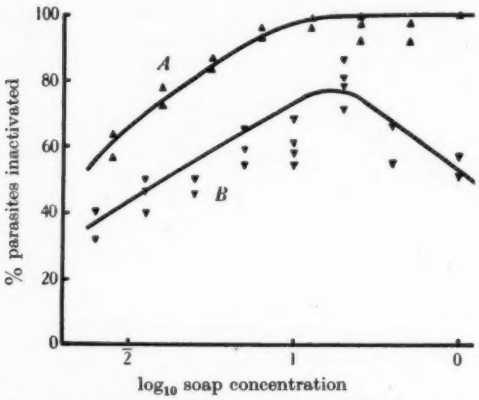

Fig. 6. Showing the effects of varying amounts of *A*, sodium laurate and *B*, sodium oleate, on the anthelmintic activity of 1/2000 hexylresorcinol in buffered saline. Soap concentration is given as the logarithm of the percentage concentration.

In all these experiments the proportion of inactive worms did not decrease when the re-examination after incubation in saline for 1 hr. was carried out. The amounts of mucus retained on the gut wall during perfusion varied considerably but appeared to be reduced in the presence of sodium laurate. Thus six tests at differing laurate concentrations showed changes in the amounts of mucus from +3·2 to −6·5 mg./cm.² (average −1·0 mg.) or +0·4 to −0·5 mg./mg. gut wall (average +0·1 mg.), and in spite of the wide range of these figures it appears that less mucus was found in the presence of laurate than when hexylresorcinol alone was used in the perfusion fluid. However, no definite relationship between sodium laurate concentration and the amounts of mucus found could be detected. Tetrachlorethylene activity was not markedly affected by the presence of sodium oleate.

The effects of bile salts on anthelmintic activity

Saline perfusion fluids, buffered as before, and containing 1/2000 hexylresorcinol, were tested at various concentrations of paired bile salts (sodium tauroglycocholate). At concentrations of about 0·03 % the anthelmintic efficiency was increased, more than 70 % of the parasites being inactivated. The perfusion fluid without tauroglycocholate inactivated from 30 to 50 % of the worms. As the tauroglycocholate concentration rose beyond 0·25 %, the proportion of parasites inactivated fell rapidly until at 2 % strength less than 10 % were affected (Fig. 8).

The changes in the amounts of mucin found on the gut wall before and after perfusion are shown in

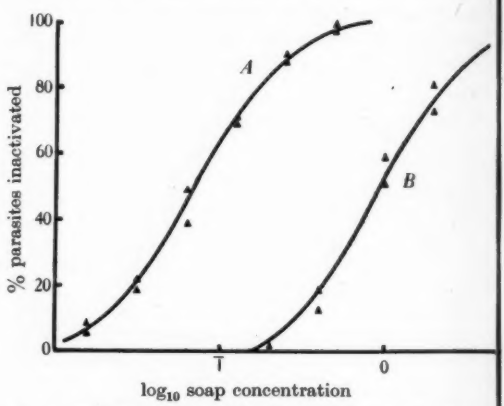

Fig. 7. Showing the anthelmintic activity of *A*, sodium oleate and *B*, sodium laurate in buffered saline. Soap concentration is given as the logarithm of the percentage concentration.

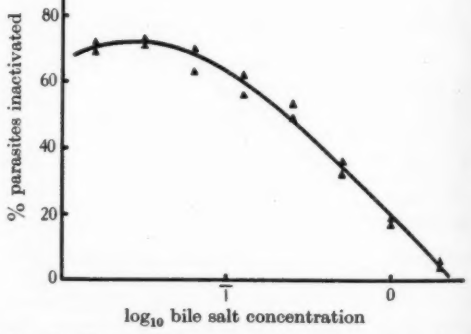

Fig. 8. Showing the effects of varying amounts of paired bile salts on the anthelmintic activity of 1/2000 hexylresorcinol in buffered saline. Sodium tauroglycocholate concentration is given as the logarithm of the percentage concentration.

Table 3. At high concentrations of paired bile salts, mucus was evidently removed from the gut wall, and this effect, at and above strengths of 0·5 %, was so marked that worms were never found embedded in mucus after perfusion. That the 'breaking' of mucus by tauroglycocholate may have had some effect on drug efficiency was shown by the fact that

the difference in the proportions of immobile worms found in the mucus and free in the intestine varied from 3 to 22% at moderate concentrations and from 12 to 37% at low concentrations. Tetrachlorethylene in moderate concentrations was not greatly affected by bile salts.

Table 3. *Showing the differences in the amounts of mucus found on the gut wall before and after perfusion with fluids containing various concentrations of paired bile salts*

Bile salt concentration %	Mucus, mg./cm.² of gut	Mucus, mg./mg. of gut
1·00	−5·4	−4·5
0·50	−9·8	−5·7
0·25	−6·8	−2·7
0·12	+4·9	+7·2
0·06	+3·8	+3·0
0·03	−1·6	+1·4
0·01	+0·7	+3·0

The effects of ascorbic acid on drug activity

Robertson, Ropes & Bauer (1940) suggested that ascorbic acid would reduce the viscosity of mucoprotein, and later McClean & Hale (1941) showed that a half-viscosity level was reached in less than 20 min. when a *M*/3000 concentration of ascorbic acid was used. As it was considered that by reducing the viscosity of the intestinal mucus hexylresorcinol might more effectively reach the parasites, perfusion tests were carried out with varying amounts of hexylresorcinol in buffered saline containing 1/2000 sodium oleate and *M*/3000 ascorbic acid. It was found that there was a slight but fairly consistent increase in drug activity (Fig. 4). Similar tests were carried out using 1/2000 hexylresorcinol and concentrations of ascorbic acid varying from *M*/750 to *M*/12,000. Except for a small peak in the region of *M*/3000, no marked changes in anthelmintic efficiency were noted, and it was considered that ascorbic acid had little effect.

IN VIVO TESTS

For reasons similar to those advanced when *in vitro* tests were discussed, *N. muris* was selected as the test organism for *in vivo* studies. Drug activity was estimated by various methods, first by means of worm and egg counts. Each test involved ten rats of a similar age to within 2 days, 6–8 weeks old, infected subcutaneously with 0·2 ml. of water containing approximately 2000 larvae. Care was taken to give each rat as nearly as possible the same infective dose. The rats were fed on a standard diet (see p. 98), and fresh faeces from each animal were collected separately during the 9th, 10th and 11th days after infection and stored in 4% formalin. Two egg counts on each sample were then carried out by the Gordon & Whitlock technique (1939). On the 12th day the animals were starved for 12 hr., and five rats, selected at random, were dosed with the appropriate drug. If large volumes were used, the drugs were passed directly into the stomachs of the lightly ether-anaesthetized rats. Small quantities were delivered into the oesophagus by means of a stout hypodermic needle blunted and rounded at the end with a blob of solder. The five control rats were similarly dosed with the vehicle, usually water, in which the drugs were given. Food was withheld for 5 hr. after dosing. Faeces for egg counting was collected on the 12th, 13th and 14th days, after which the animals were killed, the small intestine opened longitudinally and pressed between two glass plates. The parasites were then counted against a white-matt illuminated background using a low-power lens.

Early experiments showed that the variation in egg counts made the interpretation of results difficult, and the pre-treatment figures only were used to predict the expected number of worms. The parasite survival rate in the treated rats was then computed by means of the egg/worm ratio found in the controls (see Tables 4 and 5).

Table 4. *Showing the anthelmintic activity of hexylresorcinol, 115 mg./kg., given in 0·1 ml. of water. 'Egg count' represents 1/600 of the total egg output during the 9th, 10th and 11th days. Infective dose was 2200 larvae*

Control		Treated	
Egg count	Worms found	Egg count	Worms found
869	990	912	769
1006	758	535	1030
506	606	1141	1141
639	800	1314	964
747	786	672	872
Worm/egg ratio 1·05		Worms predicted 4900	
		Worms surviving 98%	

Table 5. *Showing the anthelmintic activity of tetrachlorethylene, 1 ml./kg. given undiluted. 'Egg count' figures are stated as in Table 4. The infective dose used was 1800 larvae*

Control		Treated	
Egg count	Worms found	Egg count	Worms found
196	542	136	98
275	828	181	72
112	479	157	240
56	436	131	73
98	502	147	94
Worm/egg ratio 3·78		Worms predicted 2842	
		Worms surviving 20%	

It can be seen that hexylresorcinol at the rate of one-fifth of the L.D. 55 dose (Lamson, Brown & Ward, 1935) did not appreciably affect the worm burden. A further test using one-quarter of the L.D. 55 dose gave a parasite survival rate of 94 %, showing no significant lowering of the number of worms. Tetrachlorethylene (Table 5), on the other hand, even in very small doses, reduced the worm population by 80 %, a highly significant change (using the *t* test of significance for small numbers). The efficiency of 0·1 ml. doses of C_2Cl_4 was not appreciably affected by 20 mg. paired bile salts given in 1 ml. of water. Sodium oleate did not appear to affect the activity of hexylresorcinol. It must be noted, however, that small changes in anthelmintic efficiency would not be detected by the method used unless large numbers of animals and elaborate statistical analysis were used (Whitlock & Bliss, 1943).

The distribution of parasites along the intestine of the rat

Standard rats of the same age were found to have the parasites distributed along the intestine in a similar manner, the bulk of the population being grouped in about 15 cm. of intestine some 20 cm. from the pyloris. When infected animals were dosed the worm population moved down the intestine.

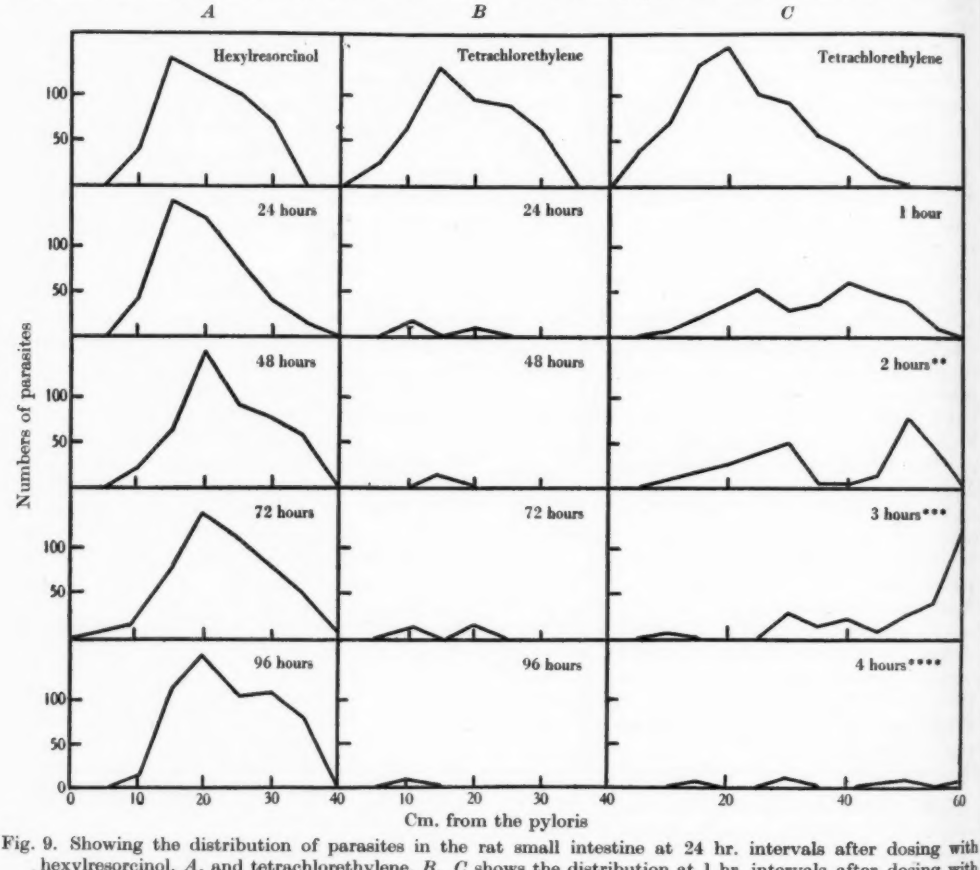

Fig. 9. Showing the distribution of parasites in the rat small intestine at 24 hr. intervals after dosing with hexylresorcinol, *A*, and tetrachlorethylene, *B*. *C* shows the distribution at 1 hr. intervals after dosing with C_2Cl_4. The distribution found in control animals and at 1 hr. intervals after dosing with hexylresorcinol were similar to *A*. * Some parasites found in the caecum.

This was used to examine the rate of action and relative activity of drugs.

Groups of standard rats were dosed with appropriate anthelmintics and killed at 24 hr. intervals. The intestines were examined between glass plates marked in 5 cm. squares and the distribution curves of the parasites determined. Results obtained with hexylresorcinol and tetrachlorethylene are shown in Fig. 9. It is clear that the former drug had no effect, whereas the latter removed large numbers of worms

within 24 hr. These experiments were repeated, rats being killed at 1 hr. intervals (see Fig. 9). Sodium oleate (20 mg.) or bile salt (20 mg.) did not cause any significant change in these results. Evidently hexylresorcinol was inactive in the rat, and in view of the results obtained from *in vitro* tests the following possible inhibiting factors were examined: (*a*) bile-salt concentration in the rat small intestine; (*b*) the rate of passage of hexylresorcinol down the rat alimentary tract; (*c*) the passage of hexylresorcinol through mucin layers.

The amounts of bile salt in the rat small intestine

Bile salt was estimated colorimetrically in the presence of laevulose and strong acid (Snell & Snell, 1937). When large amounts of hexylresorcinol were present, samples were washed with 50/50 petrol ether-benzene before estimations were carried out. Rats (starved overnight) were killed with ether at intervals after dosing them with 5 mg. hexylresorcinol and 5 mg. sodium oleate in 5 ml. of water (2·5 ml. in the case of rats 4 and 5, see Table 6). The 1st 40 cm. of small intestine was removed and the contents washed out with 25 ml. of water. Some difficulty was experienced in expressing all water from the intestinal lumen and the estimation of the volumes of fluids initially present was only approximate. When bile-salt concentrations were being determined, intestinal contents were centrifuged and known volumes taken from the supernatant fluid for examination (rats 1, 2, 3 and 7, see Table 6). Table 7 shows the amounts of bile salt found in fasting infected and uninfected rats.

The passage of hexylresorcinol down the rat intestine

Hexylresorcinol was estimated colorimetrically with *p*-amino benzoic acid (Trim, 1943). Since hexylresorcinol adsorbed on mucin probably does not penetrate the nematode cuticle (Trim, 1943), only that anthelmintic present in the lumen of the alimentary canal (i.e. that which could be washed free with water) was determined except when the retention of the drug in the stomach was being examined, in which case it was extracted with 2% NaOH. Thus rats which had been starved overnight were killed at varying intervals after being dosed with 5 mg. hexylresorcinol and 5 mg. of sodium oleate in 5 ml. of water. The contents of the stomach and the 1st and 2nd halves of the small intestine were extracted with water and the drug present determined. Finally, the drug content of the alkali extract of the stomach mucus and food particles (if present) was estimated. Results are given in Table 8. Similar experiments were carried out after giving more concentrated doses of hexylresorcinol with and without $NaHCO_3$. Drug retention in the rat stomach was not affected by these measures.

Table 6. *Showing the amounts of bile salt found in the 1st 40 cm. of rat small intestine or in samples of known volume taken from that region (rats 1, 2, 3 and 7). For further explanations see text (p. 105)*

Rat no.	Time after dosing min.	Volume (ml.) of intestinal contents	Bile salt mg.
1	Fasting	0·30	1·85
2	30	0·12	1·56
3	30	0·10	1·03
4	30	0·6	4·2
5	30	0·2	2·26
6	30	0·9	3·90
7	60	0·50	3·92
8	60	0·6	1·98
9	60	0·4	4·95
10	90	0·2	1·86
11	90	0·3	2·40
12	120	0·4	1·80
13	180	0·3	2·91

Table 7. *Showing the amounts of bile salt found in the 1st 40 cm. of the small intestines of infected and uninfected rats*

Bile salt (mg.)	
Infected rats	Uninfected rats
5·02	1·19
5·41	1·13
4·26	3·88
4·10	2·54
3·80	3·34

Table 8. *Showing the amounts of hexylresorcinol found after dosing rats. 'Stomach sediment' refers to that obtained from stomach mucus by extracting with NaOH. In the first two cases drugs were given in 2·5 ml. of water, in the remainder, 5 ml. was used. For further explanation see text*

	Hexylresorcinol content in mg.			
	Small intestine		Stomach	
Time in min.	1st 40 cm.	Remainder	Washings	Sediment
30	0·75	—	0·41	3·41
30	0·64	—	0·26	2·72
30	0·37	—	0·46	2·01
30	0·66	—	0·18	2·83
60	0·47	—	0·25	2·10
60	0·54	—	0·14	2·82
90	0·22	0·21	0·41	2·04
90	0·20	0·09	0·24	1·86
120	0·12	0·02	0·04	3·10
180	0·03	0·06	0·07	1·83

The diffusion of hexylresorcinol through mucin

This was determined by Oeholm's modification of the von Wagau sliding cell method using Stefan's tables (for references and a review of methods see Williams & Cady, 1934). As a test of technique, diffusion constants for urea were determined in the same experiments. Exps. 1 and 2 (see Table 9) were carried out using 1·25 % mucin (prepared from dry 'gastric mucin' of Armour and Co.) in 0·13 *M* phosphate buffer at *p*H 6·5. Mucin from the washed rat intestine, in 0·9 % NaCl buffered with isotonic phosphate to *p*H 6·5, which had been dialysed against the saline, was used in Exps. 3 and 4. On a dry-weight basis, making allowance for added salt, this gave a concentration of 3·5 % mucin. In all cases, the mucin was lightly centrifuged before use. Hexylresorcinol was estimated by Trim's (1943) method, the amounts found in 'control' cells containing drug and mucin, and 'blank' cells containing mucin only, being determined besides that in the experimental cells. Diazotization sometimes gave an appreciable colour in the 'blank'. Urea was estimated by Cole's method (1937). Results are shown in Table 9.

Table 9. *Showing the diffusion of urea and hexylresorcinol* (18° C.) *through mucin determined in the same group of cells for each experiment. For further explanation see text. Each cell held approximately* 1 *ml.*

	Exp. 1. 18° C., 21 hr.		Exp. 2. 18° C., 17 hr.	
	Hexylresorcinol mg.*	Urea mg.	Hexylresorcinol mg.*	Urea mg.
Cell 1	0·069	4·89	0·070	0·93
Cell 2	0·101	5·83	0·109	1·08
Cell 3	0·155	7·66	0·145	2·00
Cell 4	0·325	14·40	0·440	3·24
Control	0·576	32·78	0·681	6·48
Weighted mean value of x	0·27	0·15	0·47	0·18
D_{18} (c.mm.² per day)	0·55	1·01	0·41	1·08

	Exp. 3. 18° C., 17 hr.		Exp. 4. 18° C., 17 hr.	
	Hexylresorcinol mg.*	Urea mg.	Hexylresorcinol mg.*	Urea mg.
Cell 1	Nil	0·43	Trace	0·16
Cell 2	Trace	0·76	Trace	0·60
Cell 3	0·110	1·30	0·162	1·05
Cell 4	0·372	1·80	0·801	1·41
Control	0·434	4·18	1·042	2·83
Weighted mean value of x	—	0·17	—	0·14
D_{18} (c.mm.² per day)	—	1·13	—	1·39

* Blank values have been subtracted.

The values obtained for D_{18} urea fall within the range of figures found in the literature, some of which are shown in Table 10. Thus convection (slight, in any case, owing to the viscosity of mucin) and vibration could not have been great. In Exps. 4 and 3, hexylresorcinol diffusion was almost nil, that found in cell 2 being partly attributable to slicing error. Failure to diffuse was probably due to the formation of hexylresorcinol-mucin micells, for, though some bile salt, which also adsorbs the drug, may have been present, the amount, after washing and dialysing, must have been small. Evidently, the ability of the mucin used in Exps. 1 and 2 to adsorb hexylresorcinol had been reduced during the preparation of the dried powder.

Table 10. *Showing published diffusion constants for urea in different media*

Urea content %	Diffusion constant in c.mm.²/day	Dispersion medium	Observer
3	0·639 at 5° C. (*approx. 0·96 at 18° C.)	Water containing 1·5 % agar	Friedmann (1930)
5	0·509 at 5° C. (*approx. 0·76 at 18° C.)	Water containing 5 % gelatine	Friedmann (1930)
3	1·410 at 18° C.	Water	Zuber (1932)
1	1·332 at 18° C.	Water	Zuber (1932)

* Values at 18° C. were calculated by means of the formula

$$D_{18} = D_5 \times 291/278 \times \eta 5/\eta 18,$$

the last term being the ratio of the viscosities of water at 5 and 18° C.

DISCUSSION

The scope of the methods used

Successful assessment of parasite mortality by the lack of response to stimuli (heat and high O_2 tensions in the present experiments) depends on the type of drug examined. Some drugs, e.g. santonin, are said to be effective without even causing generalized paralysis, the evacuated worms being active and apparently healthy. In such cases motility tests are useless. However, the action of hexylresorcinol and C_2Cl_4 on *N. muris* was marked and clear-cut. Further, the perfusion method has been used to examine the conditions governing anthelmintic activity rather than to determine the possible field value of different drugs. For the detection of new anthelmintics the method has limita-

tions, drugs active in perfusion not necessarily being active in the live animal though it is unlikely that drugs which are inactive in the perfused gut would be efficient in the live animal.

The behaviour of worms in the perfused gut

The position of the worms in the perfused gut was not greatly affected by hexylresorcinol and, except when the drug was used in high concentrations of bile salts, few parasites fell into the lower cannula. Even hyperperistalsis, induced by increasing the osmotic pressure of the bath medium, did not appear to displace the worms, although the large volumes of fluid passing through the gut loosened the surrounding mucus. Tetrachlorethylene, on the other hand, evidently freed the parasites from mucus or stimulated them to enter the mobile fluids in the intestinal lumen from whence they fell into the lower cannula when killed. Thus, after perfusion with C_2Cl_4, large numbers of worms, roughly proportional to the total numbers of inactive worms found, could be drawn off from the lower cannula. Similarly, in the rat large numbers of worms were found moving freely down the intestine after dosing. Not all parasites killed by C_2Cl_4 or CCl_4 were passed immediately, however, for some remained in the mucus and were digested *in situ* some 48 hr. later. (Slight digestion was also noted in worms in the intestinal lumen 3 hr. after treatment.) The stimulation of *N. muris* to leave mucus by C_2Cl_4 or CCl_4 may be similar to that (Findlay, 1939) inducing *Ascaris lumbricoides* to migrate in the treated host animal.

The rate of action of the anthelmintics

Having gained contact with *Nippostrongylus muris*, both C_2Cl_4 and hexylresorcinol probably acted rapidly. The relative fall in the killing rate after longer periods (see Figs. 2, 3) appeared to be due to the protection afforded by mucus to a proportion of the worms. When given to the rat in concentrated doses, C_2Cl_4 acted rapidly, a considerable number of worms being affected within an hour (see Fig. 9) and the drug, moving at a rate similar to that of the water-Ba meal, was frequently seen some 60 cm. from the pyloris after 2 hr. Clearly, anthelmintics efficient in the short active intestine of the rat must kill rapidly or at low concentration. Thus phenothiazine, which perfusion studies show to be a relatively slow-acting anthelmintic and which is known to be efficient only in high concentrations, cannot effect *N. muris* in the rat unless artificial means of increasing its killing rate or delaying its passage along the intestine are used. If effective concentrations of a drug pass the parasites in the rat intestine in 1 hr. and take 6 hr. (Dr Phillipson, private communication) in the sheep intestine, such drugs, though effective in sheep, may be inefficient in the rat.

Bile salt and anthelmintic activity

Trim (1943) has shown that 1% paired bile salt completely inhibits the passage of hexylresorcinol through *Ascaris lumbricoides* cuticle. Similar tauroglycocholate concentrations reduced drug efficiency against *Nippostrongylus muris* by 50% (Fig. 8). Clearly, if bile salt reaches high concentrations in the small intestine, its inhibitive action will be important *in vivo*. Dopter & Deschiens (1938) quote Chabrol (no reference given) as finding 1·5% bile salt in human duodenal fluid. In the rat, varying concentrations from 0·6% (fasting animals) to 1·3% (30 min. after dosing) were found (Table 6). Since infected rats appeared to pass more bile salt (Table 7), and because ether, which depresses bile secretion (Steinmetzer, 1926), was used when dosing and killing the animals, these figures may be low. With larger doses the level may have been higher for soap and resorcinol stimulate hepatic secretion (Chabrol, Charonnet, Maximin & Bocquentin, 1930). In the rat, therefore, it may be considered that the therapeutic dose of hexylresorcinol and sodium oleate may become 50% inhibited by bile salt. If the activity of an anthelmintic could be maintained in the presence of bile salt, the mucus dispersing function of the latter (see p. 103) might increase its efficiency.

Detergents and anthelmintic activity

Rising concentrations of sodium oleate given with hexylresorcinol affected anthelmintic activity in three stages: (1) 0·01–0·2%, surface activation of hexylresorcinol (Fig. 6), (2) 0·2–1·0%, hexylresorcinol inhibited (Fig. 6), and (3) 1% upwards, soap alone killing *N. muris* (Fig. 7). Trim (1943) found that low concentrations of oleate increased the rate of passage of hexylresorcinol through *Ascaris lumbricoides* cuticle while high concentrations reduced it. Evidently similar actions took place in both parasites. Sodium laurate was more active than oleate at *p*H 6·5 and the inhibitory effect was masked owing to the intrinsic toxicity of the soap at low concentrations (see Figs. 6, 7). At lower H^+ concentrations the activity levels of oleate and laurate are reversed (Jarisch, 1922) and hence oleate may be more efficient against parasites in alkaline media.

Having considered the *p*H of the region where anthelmintic action is required and the precipitation of soaps by Ca^{++} in the intestine, the examination of a series of such compounds seems desirable.

The protective action of mucus

Although the conditions of the perfusion experiments were such as to loosen the mucus surrounding the parasites, the protective action was still evident (see p. 101). In the rat, *Nippostrongylus muris* was found deep in mucus between the villi, and as the parasites themselves seemed to stimulate mucus secretion, the protection obtained must have been great, and indeed hexylresorcinol was ineffective. Adsorption of the drug on mucin probably caused this. Occurring first in the stomach (see p. 105) adsorption prevented the drug reaching the intestine in high concentration and the small amounts which passed slowly into the duodenum were again adsorbed on intestinal mucus. Clearly, the concentration in the intestinal lumen seldom rose to toxic levels and the inability of the hexylresorcinol-mucin micell to perfuse through to the parasites coupled with its poor penetration of nematode cuticle (Trim, 1943) led to the loss of activity in the rat.

Reducing the adsorption of hexylresorcinol would lead to increased activity. Thus retention in the stomach, which takes place even in alkali (see p. 105) could be overcome by 'enteric' coatings such as phenyl salicylate (unfortunately an hepatic stimulant) dissolving in duodenal fluids. The reduction of gastric and intestinal mucus by atropine and, though ascorbic acid failed in this respect, the lowering of mucus viscosity may assist anthelmintic action. Lastly, food which not only adsorbs the drug (Lamson, Caldwell, Brown & Ward, 1931) but also increases bile and mucus secretion (see p. 100) should be withheld for a period before and after dosing.

In view of the marked inhibition of hexylresorcinol by mucin and bile salt its success as an anthelmintic is surprising. Although this drug attacks the cuticle of *Ascaris lumbricoides* (Lamson & Ward, 1932) in certain regions of the intestine, it would appear that ingestion of the adsorbed anthelmintic with mucus may at least constitute a supplementary mode of entry into the parasite. The fact that *A. lumbricoides* may be passed up to 10 days after treatment (Brown, 1934) lends support to this suggestion. The failure of treatment *per os* against *Enterobius vermicularis* (Brown, 1932) was probably due to the presence of bile, mucus and food residues, the few worms passed probably being killed by ingesting hexylresorcinol-mucin micells. (*Oxyuris equi* is known to ingest mucus, Wetzel, 1931.) On the other hand, enema treatment, where the conditions for hexylresorcinol activity were similar to those used in the perfusion experiments, was successful.

The relative importance of inhibiting factors compared with the degree of toxicity to parasites indicates that substances similar to hexylresorcinol, which, though less toxic to nematodes, are not inhibited by mucin and bile salt, may be valuable anthelmintics. The wide range of activity of C_2Cl_4 may be attributed to its relative therapeutic stability in gastric and intestinal fluids, the variants bile and mucus having little effect on the drug.

SUMMARY

1. A method for examining conditions governing anthelmintic activity in the small intestine is described. *Nippostrongylus muris* in the rat was the test organism and hexylresorcinol and C_2Cl_4 were the drugs examined.

2. The efficiency of hexylresorcinol was reduced by 50% in 1% sodium tauroglycocholate which reached a concentration of 1·3% in the rat small intestine. The adsorption of this drug on mucin reduced its concentration in intestinal fluids and prevented penetration to parasites under mucus. These inhibiting factors account for the inactivity of hexylresorcinol in the rat.

3. Tetrachlorethylene, which stimulated *N. muris* to leave mucus and enter fluids in the intestinal lumen, was not inhibited by bile salt and was found to act rapidly in the rat.

4. Sodium laurate activated hexylresorcinol more efficiently than sodium oleate at *p*H 6·5. Both soaps showed intrinsic anthelmintic properties.

5. The results obtained in examining the rate of movement of fluids down the intestine and the effects of detergents, bile salts, mucin and ascorbic acid on drug activity are discussed.

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DIRECT AND INDIRECT ACTIONS OF RADIATION ON VIRUSES AND ENZYMES

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(With 4 Figures in the Text)

INTRODUCTION

Work in recent years on chemical and biological actions of X-rays and radioactive radiations has included studies of the inactivation by these agents of viruses and enzymes. The impression given by the literature is that the mode of action of radiation is different in the two cases. Virus inactivation has almost invariably been interpreted as due to the dissipation of energy, by ionization, inside the virus particle itself. Evidence has been collected suggesting that a *single* ionization inside the virus particle suffices to cause inactivation. On this basis it has been proposed that measurement of the dose of radiation required to inactivate a given virus can be used to distinguish between the 'macromolecular' type of virus, of which the crystallizable plant viruses are the best examples, and the viruses of more complex structure, such as vaccinia, which are more akin to single-celled organisms (Lea & Salaman, 1941). It has further been proposed that in the case of the macromolecular type of virus, the determination of the inactivation dose can be used as an approximate method of determining the size of the virus (cp. Wollman & Lacassagne, 1940; Wollman, Holweck & Luria, 1940; Lea, 1940*b*; Gowen, 1940; Luria & Exner, 1941; Exner & Luria, 1941; Lea & Smith, 1940, 1942).

Turning now to enzymes, the recent experiments of Dale (1940, 1942; also Dale, Meredith & Tweedie, 1943) on the inactivation by X-rays of aqueous solutions of purified enzymes have shown convincingly that the effect of the radiation is indirect. To inactivate a molecule of enzyme in these experiments it was not necessary for energy to be dissipated by the radiation in the molecule itself. Energy dissipated in the water by the radiation could lead to the inactivation of the enzyme, presumably by the intervention of an intermediate agent formed by ionization or excitation of the water. Indirect actions of this sort have for some time been recognized in the study of chemical changes induced by X-rays in aqueous solutions of various organic and inorganic substances. The intermediate agent is usually known as *activated water*, its actual nature however being unknown and difficult to investigate since it has only a transient existence (cp. Fricke, 1934, and other papers by this author).

In Dale's experiments on enzymes (as also in those of Fricke on simpler solutes) the inactivation dose was not a constant characteristic of the enzyme, but depended on the concentration of the enzyme in the solution, being smaller for dilute than for concentrated solutions, and over the range of concentration investigated was directly proportional to concentration. The inactivation dose was increased when to a dilute solution of the enzyme a protective agent was added. Numerous organic, and some inorganic compounds acted as protective agents, simple compounds like NaCl or phosphate buffers however having no effect. Under these circumstances it is obvious that it is not possible to use the inactivation dose to obtain an approximate estimate of the molecular weight of the enzyme in the manner proposed for viruses, and an absurdly high figure would in fact be obtained if this were attempted.

The object of the experiments described in the present paper was to decide whether enzymes and viruses are fundamentally different in the mechanisms of their inactivation by radiations, as suggested by a superficial study of the findings we have briefly reviewed. The experiments of Luria & Exner (1941) suggest that the difference may not be fundamental, since they find evidence for an indirect effect of radiation on phages under certain circumstances. Also Friedewald & Anderson (1940, 1941) find evidence of an indirect effect on the Shope rabbit papilloma virus. The conclusion we have reached from our experiments is that the mechanism of inactivation is very similar in the two cases. Irradiated dry, the inactivation is direct, due to the production of a single ionization in the virus particle or enzyme molecule. From the inactivation dose

can be calculated a rough estimate of the size of the virus or of the molecular weight of the enzyme. In dilute solution many more ionizations are produced in the water than in the virus or enzyme, since energy absorption is approximately equal for equal masses of solute and solvent, and inactivation is there mainly indirect. If an ionization in the water were as effective in causing virus inactivation as an ionization in the virus particle itself, then in a 10% solution of virus in water the inactivation dose would be 10 times smaller than the inactivation dose of the dry virus, since to every ionization in the virus itself 9 additional ionizations occur in the solvent. However, it appears that an ionization in the water has a rather small probability of causing inactivation of the virus, presumably because the activated water can lose its energy on contact with a virus particle without invariably leading to inactivation of the virus particle. Thus a 10%, or even a 1% virus solution shows an inactivation dose practically as high as dry virus, and only at higher dilution does the indirect effect become noticeable.

The presence of a substance such as gelatin or probably of any protein provides an alternative means of deactivating the activated water as effective as the virus itself, and hence it is only when the *total* protein content of the solution is well below 1% that indirect inactivation of the virus becomes noticeable. It is for this reason that the indirect action is liable to be overlooked. When a crude virus preparation is employed, we are often not able to detect the virus at a dilution high enough for the indirect action to be noticeable. If a purified virus preparation is irradiated at high dilution, however, an indirect effect is found. Most of the experiments reported in the literature on the inactivation of viruses by radiation have not employed highly purified virus preparations, and thus even when they have been performed in solution it is correct to interpret them in terms of direct action. To be quite safe in this interpretation it is of course advantageous to make the irradiation on dried virus preparations.

As regards enzymes, the dose of radiation required for their direct inactivation is so great that experiments on crude preparations or in concentrated solution have in the past usually failed to show any inactivation at all. By employing very high doses we have been able to follow the direct inactivation of dried enzymes. Interpreting these results in the same manner as has commonly been used in determining the sizes of viruses from their inactivation doses, we are led to estimates of the molecular weights in fair agreement with the probable values. The radiation method may perhaps occasionally be of value in giving a rough estimate of the molecular weight of an enzyme not available in sufficiently pure form for more exact methods to be employed.

EXPERIMENTAL

Direct inactivation of enzymes

The enzymes employed in studying the direct inactivation by dry irradiation were ribonuclease and adenylpyrophosphatase (i.e. myosin). The methods of preparation and estimation of the enzymes were as follows. Myosin was prepared by the method described by Bailey (1942), being twice precipitated from water. It was dried in a desiccator after the addition of 0·5% of lactose (Svedberg & Pedersen, 1940) to the wet jelly. Preparations dried in this manner were sufficiently active to make possible the estimation of small amounts of enzyme. The myosin was estimated as adenylpyrophosphatase by the method of Needham (1942), the adenosine triphosphate being prepared according to the method described in the same paper.

Amounts of about 2 mg. dry weight were taken for the irradiation and were weighed in the dry form after the irradiation, the whole amount irradiated being used. This was made necessary by the fact that small dry flakes did not redissolve sufficiently to give a homogeneous solution. Over the range used the relationship between the enzyme concentration and the amount of inorganic phosphate appearing was nearly linear.

Ribonuclease was crystallized, recrystallized, and estimated according to the methods of Kunitz (1941). The activity of the preparation was far less than that quoted by Kunitz. This may have been due to the fact that large amounts of ox pancreas were not available and crystallization of the small amounts of enzyme obtained was always slow. It was possible to dry the enzyme in a desiccator (after the addition of 0·5% of lactose to the solution) with the loss of about half of its activity.

Two separate preparations were employed. In each case the determination of activity gave some difficulty since the activity of the enzyme was considerably increased by dilution. In order to obtain a reasonable estimate of the proportion of enzyme destroyed by each dose of X-radiation, it was necessary to estimate several dilutions of each control and each irradiated sample. The ratio of the weights of control and irradiated enzyme which produced equal breakdown of ribose nucleic acid was taken as the proportion of the enzyme activity surviving irradiation.

Very high doses of X-rays were needed, and were obtained by employing an X-ray tube in which a single metal foil served both as target and window, thus permitting the specimen to be placed very close to the target. A distance of 3 mm. was used in the present experiments, the dose-rate at this distance being 2×10^6 roentgen per hour. The radiation from this tube, which operated at 40 kV., 1 mA., was

nearly monochromatic of wave-length 1·5 A. The construction of the tube, and the method of measuring the radiation from it have been described (Lea, 1941). The target end of the tube was water cooled, but owing to the low penetrating power of the radiation it was not possible to have cooling water between the target and the specimen. The energy dissipated by the target was, however, not very great (40 W.) and precautions were taken to minimize any rise of temperature of the specimen during irradiation. About 2 mg. of the powdered enzyme preparation was pressed into a shallow cylindrical brass box 4 mm. in diameter and 0·5 mm. deep, the back of which was water cooled, and the front of which, through which the radiation entered, was covered by a thin aluminium foil. The space between the target and the specimen was evacuated. Under these conditions the temperature rise of the enzyme during irradiation must have been very slight. To make sure that the inactivation obtained was in fact radiation inactivation and not thermal inactivation, some exposures were made running the tube at 20 kV. and 2 mA., i.e. half the kilovoltage and twice the milliamperage normally employed. The heat dissipated by the target by this procedure was unchanged, but the output of X-radiation was reduced approximately fivefold. The points obtained by this procedure lay on the same curve as the remaining points when the proportion of enzyme activity surviving was plotted against the dose of radiation, thus verifying that the degree of inactivation is determined by the dose of radiation and is not a thermal effect. The points obtained at reduced kilovoltage in this way are shown as crosses in Figs. 1 A, B. Control samples of enzyme were placed in the evacuated chamber during the exposures, shielded from the X-rays.

In Fig. 1 the natural logarithm of the surviving activity is plotted against the dose of radiation. The points are seen to lie reasonably well on a straight line, as is to be expected for a monomolecular reaction, and as has been found also for viruses. The inactivation dose is conveniently defined as the dose reducing the natural logarithm by unity, i.e. the dose reducing the activity to $e^{-1} = 0{\cdot}37$ of its initial value. The inactivation doses are given in Table 1.

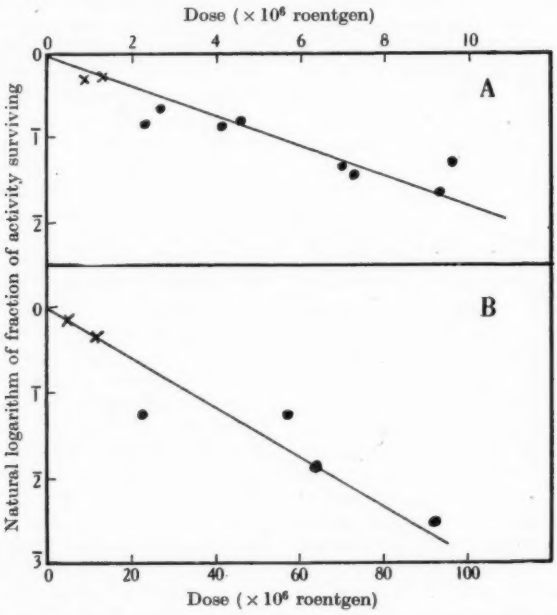

Fig. 1. Inactivation of enzymes by X-rays (1·5 A.). A, myosin; B, ribonuclease.

Table 1. *Inactivation doses of enzymes*

Ribonuclease	$3{\cdot}4 \times 10^7$ roentgen
Myosin	$5{\cdot}5 \times 10^6$ roentgen

Direct and indirect inactivation of tobacco mosaic virus

We have made a series of measurements of inactivation doses of tobacco mosaic virus irradiated by γ-rays of radium either dry or dissolved to various

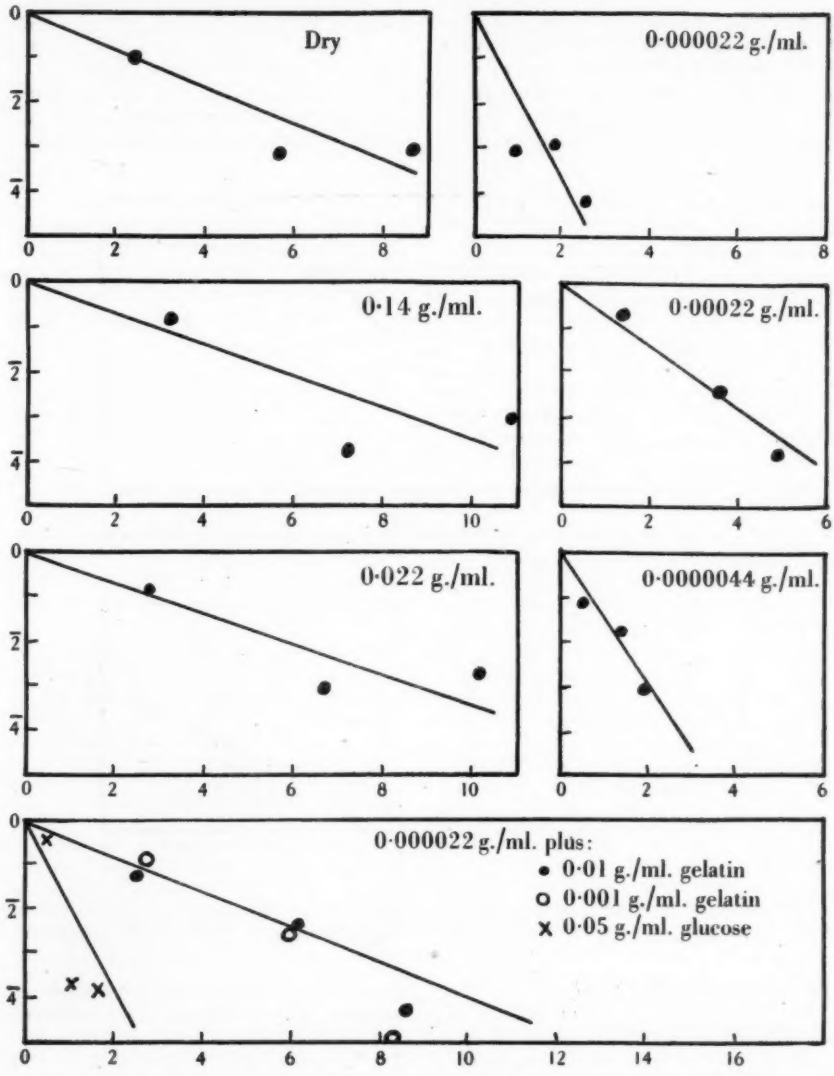

Fig. 2. Inactivation of tobacco mosaic virus in solutions of various concentrations. Abscissae: doses in units of 10^5 roentgen. Ordinates: natural logarithms of ratio of exposure: control lesion counts.

concentrations in *M*/50 phosphate buffer, with or without protective agents. The methods of irradiating, and of assessing virus activity by the lesion-counting technique, were similar to those used previously (Lea & Smith, 1940, 1942). In the present series of experiments a total of some fifty different irradiations were made, involving the counting of some 28,000 lesions on some 500 leaves of *Nicotiana glutinosa*.

A single preparation of tobacco mosaic virus was used throughout the experiments. This preparation was purified by high-speed centrifugation and contained 0·022 g./ml. of virus protein. The activity was such that to obtain a suitable number (10–100) of

lesions per half leaf a dilution of 1000–10,000 times was required. For dry irradiation, a single drop of the standard solution was put on to a coverslip and dried at 37° C. For the irradiations at concentration 0·14 g./ml. the standard solution was evaporated in a cellophane bag at room temperature to the required degree. For the irradiations at higher dilutions the standard solution was diluted in $M/50$ phosphate buffer. Irradiation took place at about 0° C. The dose rate was 7700 roentgen per hour and the exposures lasted from 6 to 150 hr. Controls were treated identically except that they were shielded from the radiation while in the refrigerator. After irradiation the specimens were diluted to a suitable degree and inoculated, half of each leaf being inoculated with the treated, and half with the control, solution. Equal numbers of left- and right-hand half-leaves were used for each sample.

In Fig. 2 we give the curves of the surviving activity (plotted as the natural logarithm of the ratio of the exposure to control lesion count) as a function of dose. Each point is the mean of two or three separately irradiated specimens, each specimen being tested on about ten leaves. From the curves we read off the inactivation dose defined as that dose reducing the logarithm by unity, i.e. reducing the lesion count of the exposure to 37 % of the lesion count of the control. These are collected in Table 2, and plotted against protein content in Fig. 3 A. It was established that irradiation of the buffer alone, followed by addition of unirradiated virus, does not lead to inactivation of the virus, showing that the 'activated water' has only a transient existence.

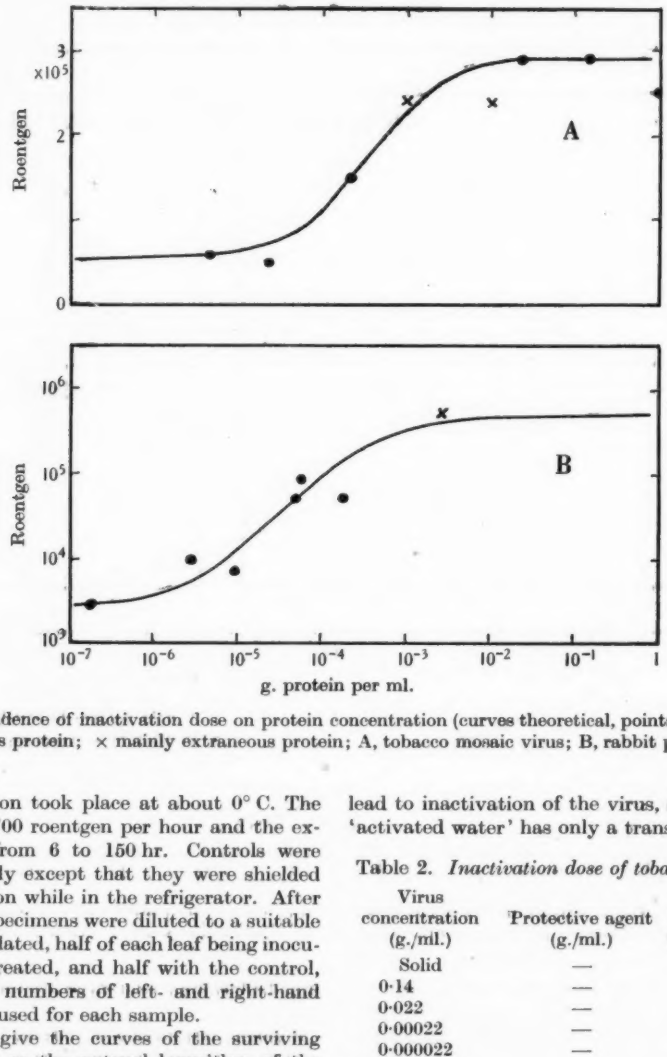

Fig. 3. Dependence of inactivation dose on protein concentration (curves theoretical, points experimental). ● mainly virus protein; × mainly extraneous protein; A, tobacco mosaic virus; B, rabbit papilloma virus.

Table 2. *Inactivation dose of tobacco mosaic virus*

Virus concentration (g./ml.)	Protective agent (g./ml.)	Inactivation dose ($\times 10^5$ roentgen)
Solid	—	2·5
0·14	—	2·9
0·022	—	2·9
0·00022	—	1·5
0·000022	—	0·5
0·0000044	—	0·6
0·000022	Glucose 0·05	0·5
0·000022	Gelatin 0·001	2·4
0·000022	Gelatin 0·01	2·4

DISCUSSION

Relation between inactivation dose and molecular weight of enzyme

As has been mentioned, experiments on virus inactivation have usually been interpreted on the basis that a single ionization in the virus particle leads to inactivation. If we interpret the experiments on the inactivation of dry enzymes in the same way, and suppose that a single ionization in the enzyme molecule suffices to cause inactivation, then it is easily seen that the inactivation dose as defined above is that dose which produces an average of one ionization per enzyme molecule. Now a dose of 1 roentgen (by definition) liberates ions carrying a total charge of 1 electrostatic unit of either sign in 0·001293 g. of air. Thus since the charge carried on an ion is $4{\cdot}8 \times 10^{-10}$ e.s.u., it follows that 1 roentgen corresponds to the production of $1/(4{\cdot}8 \times 10^{-10} \times 0{\cdot}001293) = 1{\cdot}61 \times 10^{12}$ ionizations per g. of air. Allowing for the slightly different mass absorption coefficients of protein and air, we deduce that 1 roentgen corresponds to the production of $1{\cdot}45 \times 10^{12}$ ionizations per g. of protein. This figure is subject to some uncertainty, since we have had to assume that the mean energy dissipation per ionization produced is the same in protein as in air, which is likely to be approximately but not exactly true.

Thus on the assumption that the inactivation dose of D roentgen corresponds to the production of 1 ionization per molecule, the weight of the molecule is evidently $1/(1{\cdot}45 \times 10^{12}\ \mathrm{D})$ g. In molecular weight units this becomes $6{\cdot}023 \times 10^{23}/1{\cdot}45 \times 10^{12}\ \mathrm{D}$, so that

$$\text{Molecular weight} = \frac{4{\cdot}16 \times 10^{11}}{\text{Inactivation dose in roentgen}}. \qquad (1)$$

The inactivation doses for ribonuclease and myosin being respectively $3{\cdot}4 \times 10^{7}$ and $5{\cdot}5 \times 10^{6}$ roentgen, we deduce for their molecular weights the values 12,200 and 76,000. The molecular weight of ribonuclease is between 12,000 and 18,000 (Neurath, 1942; Bridgman & Williams, 1942; Rothen, 1942), that of myosin is uncertain.

The calculation we have given relating inactivation dose to molecular weight is over-simplified inasmuch as it neglects the fact that the ionizations produced by X-rays are not randomly distributed but are localized along the paths of the photoelectrons liberated in the substance irradiated, sometimes singly, sometimes in clusters of two or three or a few ionizations. In consequence it may happen that more than one ionization is produced in a molecule by a photoelectron which passes through it. The question arises whether when two ionizations are produced simultaneously in one molecule, only one molecule is inactivated, or whether the effect of an ionization can spread so that an adjacent molecule which has not itself been ionized will in such an instance be inactivated as well as the molecule which has been ionized. There is no information regarding this point for molecules as small as ribonuclease. For viruses, there is an indication that spreading of the effect outside the molecule does not occur, for with viruses densely ionizing radiations such as α-rays which, if they ionize the virus particle at all, usually leave several ions in it, are less effective per ionization than less densely ionizing radiations such as X-rays or γ-rays (Lea & Smith, 1942). We shall provisionally assume therefore that no spreading of the effect of an ionization to an adjacent molecule which has not been ionized can occur. (It should be possible to test this point for enzymes by comparing the efficiency per ionization of α-rays and X-rays.)

The tendency for ionizations to occur in clusters will, in the absence of a spreading effect, lead to the inactivation dose being higher than it would if ionizations occurred at random, since when a cluster of several ionizations is formed in a molecule, the extra ionization adds to the dose without increasing the number of molecules inactivated. It is possible to allow for this effect and to derive a relation between molecular weight and inactivation dose (by a method described by Lea (1940*a*) and Lea & Smith (1942)). The relation is necessarily more complicated than equation (1) and is given graphically in Fig. 4, which permits the molecular weight to be read off for any given inactivation dose obtained either with X-rays (1·5 A.) or with γ-rays. We find in this way that the molecular weights of ribonuclease and myosin are 30,000 and 230,000. The value for ribonuclease agrees less well with the accepted value than did the value derived from equation (1). Possible uncertainties in the experimental determination of the inactivation dose, and in the theoretical conversion from roentgen to ionizations per g. in protein are such that it would be unwise to assume from this that spread of the effect of an ionization does occur.

It is evident, however, that whether equation (1) or Fig. 4 be used to relate inactivation dose to molecular weight, the value obtained is of the right order, and confirms that the method of calculation based on the idea that a single ionization anywhere in the molecule can cause inactivation gives the molecular weight to the right order.

As regards the molecular weight of myosin, it is to be observed that in solution myosin is rod-shaped, and the molecule defined as the minimum unit having enzyme activity may be smaller than the average particle size in solution. The estimate based on the radiation result would refer to the smallest unit having enzyme activity.

Direct and indirect inactivation of viruses

Fig. 3 A shows how the inactivation dose of tobacco mosaic virus depends on the protein content

in the solution irradiated. The principal characteristics of this curve are, that at high concentrations the inactivation dose is independent of concentration; as the concentration is reduced the inactivation dose also diminishes; and at low concentration the inactivation dose again probably becomes independent of concentration. The addition of gelatin to a dilute solution of the virus raises the inactivation dose to a value as high as in a concentrated virus solution. On the other hand glucose appears to have no protective action. This was found also by Luria & Exner (1941) studying bacteriophage inactivation.

It seems probable that the type of behaviour shown in Fig. 3 A holds for other viruses. We have been able to derive a similar curve (Fig. 3 B) for an animal virus (the Shope rabbit papilloma virus) from an analysis of the data of Friedewald & Anderson (1941) on the inactivation by X-rays of purified preparations of this virus. Tables IX and X of their paper give the percentages of inactivation produced by various doses of radiation in solutions containing five different concentrations of virus protein, and from these data the inactivation dose (defined as the dose reducing the activity to 37%) can be deduced for these five concentrations. Their Table V indicates that in solutions containing extraneous protein at a concentration of 2–4 mg./ml. the dose required for a given degree of inactivation is five times as great as with a virus concentration of 0·12 mg./ml. This furnishes the inactivation dose for a high protein concentration, namely 5×10^6 roentgen, a value agreeing with the value $4{\cdot}5 \times 10^6$ roentgen deduced from an inactivation curve obtained by Syverton, Berry & Warren (1941) for a preparation of the same virus not freed from extraneous protein. The figure 5×10^5 roentgen probably closely approaches the inactivation dose for direct effect alone. Table VII of Friedewald & Anderson's paper suggests that in a purified solution containing 0·05 mg./ml. of protein, the dose required for a given degree of inactivation is about one-tenth as great as in a solution protected against the indirect effect by addition of rabbit serum. This observation furnishes a seventh point for our Fig. 3 B.

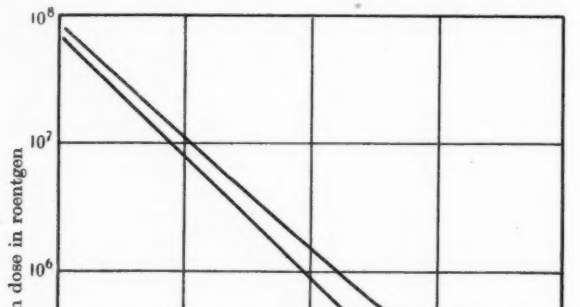

Fig. 4. Theoretical relation between inactivation dose and molecular weight.

As regards the interpretation of the shape of the curve relating inactivation dose to protein content, we have indicated in the introduction the explanation offered for the inactivation dose being independent of concentration at high concentration and then diminishing with reduction of concentration, and we can readily make the explanation quantitative. Suppose the solution contains q g. of virus per g. of water, that γ molecules of virus are inactivated per ionization produced in the water, and that Γ molecules of virus are inactivated per ionization produced in the virus itself. γ and Γ may be termed the *ionic yields* for indirect and direct action. Γ is likely to be unity or somewhat less, γ appears to be considerably less than unity. Neglecting the

slight difference in the number of ionizations per gram produced by 1 roentgen in protein and water, suppose that a dose is given which corresponds to the production of x ionizations per gram. Suppose that 1 g. of virus contains N molecules. Then in 1 g. of dry virus $x\Gamma$ out of the total of N molecules will be inactivated, namely a fraction $x\Gamma/N$. In $(1+q)$ g. of a solution containing 1 g. of water to q g. of virus $x(\gamma+q\Gamma)$ molecules of virus will be inactivated out of a total number qN, i.e. a proportion

$$(x\Gamma/N)\,(1+\gamma/\Gamma q).$$

Thus the proportion inactivated in solution by a given dose is greater than the proportion inactivated in the dry state by the factor $1+\gamma/(\Gamma q)$. Hence

$$\frac{\text{Inactivation dose dry}}{\text{Inactivation dose in solution}} = 1+\gamma/(\Gamma q). \qquad (2)$$

It is evident that with a value of γ/Γ less than unity this equation will predict an inactivation dose independent of concentration q at high concentrations, but diminishing at lower concentrations. At sufficiently low concentrations the inactivation dose should be directly proportional to concentration as found for enzymes by Dale and for a number of compounds by Fricke.

It is seen from Fig. 3 that at low concentrations the inactivation dose does not continue to diminish indefinitely but tends to a limiting value. Such an effect was found by Fricke, Hart & Smith (1938) in studying the radiation decomposition of simple organic compounds in aqueous solution and attributed by them to the activated water having a finite lifetime. In dilute solutions an appreciable time will elapse between the ionization of a water molecule and the collision of the activated water molecule with a solute molecule. If the activated water is short-lived, as appears to be the case, then in dilute solutions there is a high probability of its suffering spontaneous deactivation before it meets a solute molecule. If τ is the mean life of an activated water molecule against spontaneous deactivation, the probability that it will deactivate spontaneously in time dt is dt/τ. The probability that it will deactivate by collision with a solute molecule in time dt is proportional to the solute concentration and will be $Zpq\,dt$, where Zq is the number of collisions per second between an activated water molecule and molecules of a solute present in concentration q g./ml., and p the probability (not necessarily unity) that an activated water molecule deactivates on collision with a solute molecule. p is a constant for any given solute, but probably varies considerably for different solutes. Z again is a constant for a given solute and depends on its molecular weight; it can be approximately calculated from the kinetic theory. Under the combined influence of spontaneous deactivation and collision deactivation the probability of deactivation in time dt will be $dt\,(Zpq+1/\tau)$, and a fraction $Zpq/(Zpq+1/\tau)$ of activated water molecules will be deactivated by collision with the solute. This may be written $q/(q+q_0)$ where $q_0 = 1/Zp\tau$; it is by this factor that the yield of the reaction is less than the anticipated yield at low concentrations. Fricke *et al.* (1938) employed this factor as a purely empirical formula to express the reduction of yield below expectation at low concentrations; we have here given a justification for a formula of this type.

Combining the factor $q/(q+q_0)$ with equation (2) we obtain

$$\frac{\text{Inactivation dose dry}}{\text{Inactivation dose in solution}} = 1+\frac{\gamma}{\Gamma}\cdot\frac{1}{q+q_0}, \qquad (3)$$

relating the inactivation dose in a solution containing q g. of virus per ml. to the inactivation dose dry.

It is easy to allow for the presence of a protective agent. If such a substance is present in concentration q_1 g./ml., then the probability that an activated water molecule will deactivate in time dt will be $dt\,(Zpq+Z_1p_1q_1+1/\tau)$, where Z_1 and p_1 are the values of Z and p applicable to the protective agent. Thus a fraction

$$Zpq/(Zpq+Z_1p_1q_1+1/\tau) = q/(q+q_0+\alpha q_1)$$

of the activated water molecules are deactivated by the virus, where $\alpha = Z_1p_1/Zp$ may be described as the deactivating efficiency per unit mass of the protective agent compared to the virus protein. Thus we obtain

$$\frac{\text{Inactivation dose dry}}{\text{Inactivation dose in solution}} = 1+\frac{\gamma}{\Gamma}\cdot\frac{1}{q+q_0+\alpha q_1}. \qquad (4)$$

When a protein is being used as protective agent it is probably safe to assume that the deactivating efficiency per unit mass is about the same as for the virus being studied. The simpler formula (3) then suffices, by q being understood the *total* concentration of protein, virus protein plus extraneous protein.

In Figs. 3 A, B the curves shown are theoretical curves drawn following equation (3), with the following values of the arbitrary constant q_0 and γ/Γ.

Table 3

Virus	q_0	γ/Γ
Tobacco mosaic	$6{\cdot}1\times10^{-5}$	$2{\cdot}6\times10^{-4}$
Rabbit papilloma	$2{\cdot}54\times10^{-6}$	$4{\cdot}6\times10^{-4}$

It is evident that the curves satisfactorily fit the experimental points. Γ, the number of virus particles inactivated per ionization in the virus particle is somewhat less than unity (Lea & Smith, 1942). We see that the ionic yield for indirect action of a virus (γ) is much smaller.

The inactivation dose of tobacco mosaic virus for

the direct effect was in these experiments $2{\cdot}9 \times 10^5$ roentgen. In previous measurements (Lea & Smith, 1942) values ranging from $2{\cdot}6$ to $5{\cdot}3 \times 10^5$ roentgen were obtained with different preparations of the virus. The relation between inactivation dose and size of virus has been discussed in the earlier paper.

SUMMARY

1. The inactivation by γ-rays of tobacco mosaic virus is studied at various concentrations. It is found that the inactivation dose is independent of concentration at high concentrations, and at low concentrations also attains a constant, but lower, value. Over an intermediate range the inactivation dose increases with increase of concentration.

2. These facts are explained on the basis that when irradiated dry or in concentrated solution the inactivation is direct and due to ionization produced inside the virus particle. At lower concentrations the inactivation is largely indirect and due to ionization of the water.

3. Gelatin added to the solution protects the virus against the indirect action of radiation.

4. Curves are given of the inactivation of dry preparations of ribonuclease and adenylpyrophosphatase (myosin) by X-rays.

5. It is shown that on the assumption that a single ionization in an enzyme molecule leads to its inactivation, measurement of the inactivation dose leads to a rough estimate of the molecular weight of the enzyme.

6. There appears to be no fundamental difference in the mechanism of radiation-inactivation of viruses and enzymes.

All irradiations were carried out at the Strangeways Laboratory. The virus was prepared and tested at the Plant Virus Station and the Molteno Institute. The enzymes were prepared and estimated at the Biochemical Laboratory. We are indebted to the British Empire Cancer Campaign for defraying the cost of the X-ray equipment at the Strangeways Laboratory.

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(*MS. received for publication* 22. I. 1944.—Ed.)

Note added in proof. The suggestion has been made (Weiss, J., 1944, *Nature, Lond.*, **153**, 748) that the 'activation' of water by radiation consists in the production in the water of free H and OH radicles. The 'spontaneous deactivation' of which we find evidence would on this view be explained as the combination of H and OH radicles to reform H_2O.

'*CAPSULARIA MARINA*' AND THE ASCARIDAE OF MARINE HOSTS

By H. A. BAYLIS, M.A., D.Sc., *Department of Zoology, British Museum (Natural History)*

Prof. T. Harvey Johnston & Miss Patricia M. Mawson (1943) have recently published a paper on ascarid nematodes from Australian marine fishes, in which they have made some rather startling proposals regarding the nomenclature of marine ascarids.

One of the worms recorded by them is a larval form, from the Australian barracouta and several other fishes, which they name *Capsularia marina* (Linn.). As a result of an extensive review of the literature dealing with larval ascarids from fishes, they appear to have satisfied themselves not only that this is the correct name for the species observed by them, but that a large number of other larval ascarids recorded from marine fishes are identical with it, and that the various names that have been given to them are synonyms of *C. marina*.

The opinion is further expressed that *C. marina* is the larval form of a parasite of Cetacea whose accepted name since 1920 has been *Anisakis simplex* (Rudolphi), and which had been known as *Ascaris simplex* since 1804. *Anisakis simplex* is therefore treated as a synonym of *Capsularia marina*, and the generic name *Anisakis* (originally used as a subgenus of *Ascaris* by Dujardin in 1845) as a synonym of *Capsularia*. Further, as a logical consequence, since *Anisakis* is the type genus of the subfamily Anisakinae, the subfamily name is changed to Capsulariinae.

These changes would be inevitable, under the existing rules of nomenclature, if it were established that the argument leading to them were sound. This, however, in the writer's view, is very far from being the case. On examination it is evident that the argument rests upon a number of assumptions, and these, to the writer at least, appear entirely unwarranted and, in the present state of our knowledge, incapable of proof. It is assumed (1) that *Gordius marinus* L., 1767, is a recognizable species (at least 'in part'); (2) that *Ascaris capsularia* Rudolphi and many other larval ascarids, recorded under different names and from a great variety of hosts, can be identified with this 'part' of *Gordius marinus*; (3) that this same 'part' of *G. marinus* is the larva of *Anisakis simplex*.

These assumptions are, however, subsidiary to, or rather parts of, a much more general assumption, which may be considered first. This is that the larval forms of certain genera of Ascaridae—in particular *Anisakis* and *Porrocaecum*—can be identified not only generically but even specifically. The adults of *Porrocaecum* can be distinguished from those of *Anisakis* by the possession of an intestinal caecum in addition to the oblong or elongate 'ventriculus' common to both genera. Prof. Johnston and Miss Mawson appear to believe that this distinction is applicable to the larval forms of these genera at all ages. They also state that the larvae of the two genera occur in different situations in their hosts—those of *Anisakis* 'chiefly in the subperitoneal and mesenteric tissues', and those of *Porrocaecum* 'mainly in muscular tissue'. The writer believes that neither of these distinctions holds good.

In a paper published in 1916, the writer (Baylis, 1916) discussed, under the name of *Ascaris capsularia*, a type of ascarid larva that is extremely common in marine fishes off the British coasts, and is found both in the intermuscular connective tissue and in the subperitoneal tissues. This larva, in its later stages, has a well-developed intestinal caecum, but it was pointed out that in smaller forms (less than 28 mm. in length), otherwise appearing to belong to the same species, the caecum was not present. In specimens over 28 mm. in length a caecum was present, at first very short, but gradually increasing in length with the size of the worms. Considerably greater experience acquired since that time has tended to confirm the writer in the belief that the caecum of this type of larva (*Porrocaecum*) is only developed gradually during its sojourn in the fish host, and that the young larvae, before its development, cannot be distinguished with any certainty from those of *Anisakis*. It is for this reason that in more recent publications (e.g. Baylis, 1929, p. 546), when recording larvae with a ventriculus but without an intestinal caecum, the writer has been careful to call them '*Porrocaecum* or *Anisakis* sp.'

If, as the writer believes, it is in many cases impossible, on purely morphological characters, to distinguish these larval forms generically, it follows that it is no less impossible, by this means, to distinguish them specifically. This being so, it is perhaps unnecessary to pursue the argument in greater detail. We may, however, without going

over all the ground again, briefly consider the subsidiary assumptions.

(1) Is *Gordius marinus* L. a recognizable species? —*Gordius marinus* was first described by Linnaeus in the 12th edition of his *Systema Naturae*, at p. 1075. The writer has not, at the moment, ready access to this edition, but in the 13th edition, published in the same year and in the main a reprint of the 12th, the following account of the species appears on the same page:

'G. plano-spirali convolutus filiformis... Habitat in Maris Norvegici &c. piscibus, frequentissime in visceribus *Clypeae halecis*, intra pustulam... Haeret semper in plano spirali, albus, laevis, pollicis ½ longitudine, apice vix attenuatus.'

The above quotation contains the whole of the descriptive matter, and probably very few modern helminthologists would accept it as a recognizable description of a species, or even of a genus. The use of the expression *Gordius marinus* Linn. 1767 '(in part)' in the synonymy of *Capsularia marina* given by Johnston & Mawson seems, in itself, to be an admission that *Gordius marinus* is a composite species, and it is not clear how that part of it is to be recognized which is regarded as typical. The only host specifically mentioned by Linnaeus is '*Clypea halecis*', and it might be argued that specimens from that host only were the genuine *Gordius marinus*. But more than one species may occur in the same host, so that this criterion is of no practical value.

(2) How is the conclusion reached that *Ascaris capsularia* Rudolphi, 1802, is identical with *Gordius marinus*? The essential steps in the argument, apparently, are as follows:

(*a*) Rudolphi's *Filaria capsularia* and his *Ascaris capsularia* are one and the same.

(*b*) Rudolphi included the *Capsularia halecis* of Zeder, 1800, in the synonymy of *Filaria capsularia*, and Zeder's *Capsularia salaris* in that of *Ascaris capsularia*.

(*c*) *Capsularia halecis* (from the herring) and *C. salaris* (from the salmon) are identical with each other, and therefore both identical with *Filaria capsularia* Rud.

(*d*) Rudolphi, in 1809, treated *Gordius marinus* as identical with his *Filaria capsularia*.

(*e*) Therefore *Ascaris capsularia* and *Gordius marinus* are identical.

No evidence appears to be offered in support of the assumptions contained in links (*a*) and (*c*) of the above chain of reasoning, and they must be regarded as matters of opinion. It is something of a mystery why Rudolphi placed in different genera two worms that appear so similar as his *Filaria capsularia* and his *Ascaris capsularia*. He states, however, that the former occurs in the herring and the latter in the salmon, and, prima facie, it does not seem very likely that they are specifically identical. It should also be pointed out that the fact that Rudolphi regarded a previously described form as identical with one of his own does not constitute proof that the species are, in fact, synonymous.

(3) Johnston and Mawson state that 'the true *Ascaris capsularia* is the larval stage of the dolphin parasite commonly known as *Anisakis simplex*'. The writer is unable to find in their paper any evidence in support of this view, which appears to be a pure assumption. Some measurements are given of four larvae from the barracouta (*Thyrsites atun*), which are assigned by the authors to *Capsularia marina* (i.e. '*Ascaris capsularia*'). These apparently had no intestinal caecum, but the largest of them measured only 28 mm. in length, and therefore (as has already been indicated) the possibility cannot be excluded that they would have developed a caecum later, and were, in fact, larvae of *Porrocaecum* sp. Even if these larvae did belong to a species of *Anisakis*, why should it be *A. simplex* rather than any other?

To sum up, the writer at least is not prepared to identify any Ascarid larva with '*Capsularia marina*', still less to accept it as proved that the latter is the larva of *Anisakis simplex*. There is, therefore, in the writer's view, no reason at present for suppressing the generic name *Anisakis* in favour of *Capsularia*, and consequently none for changing the name of the subfamily Anisakinae.

Later in their paper Johnston & Mawson propose to change the name of the well-known seal-parasite *Porrocaecum decipiens* (Krabbe, 1878) to *P. piscium* (Rud., 1809), on the ground that Dujardin (1845), under the name of *Filaria piscium* Rud., described a worm which had the characters of a *Porrocaecum* larva. It is true that Dujardin describes an intestinal caecum as being present in his worm, but there is no mention of such a structure in Rudolphi's original account of *Filaria piscium*. Moreover, Dujardin includes in his synonymy of this species *Gordius marinus* L., *Capsularia halecis* (Gmel.) Zeder and *Filaria capsularia* Rud., all of which, according to Johnston & Mawson, are identical with the larva of *Anisakis simplex*, and following their system of reasoning, *Filaria piscium* Rud. should be another synonym of *Capsularia marina*. The writer is, therefore, unable to agree that a case has been made out for changing the name *Porrocaecum decipiens* to *P. piscium*.

Various authors have claimed to be able to identify specifically larval stages of Ascarids in marine fishes. Thus Wülker (1930) records larval forms from numerous fishes, particularly Gadidae, from the North Sea, which he considers to be those of *Anacanthocheilus rotundatus*. Judging by his figures, however, there seems to be nothing to

distinguish these larvae from those of *Anisakis* or from young larvae of *Porrocaecum*. Kahl (1938, 1939) believes that he can identify the larvae of *Porrocaecum decipiens*, *Anacanthocheilus rotundatus* and *Contracaecum clavatum*, but does not mention the morphological criteria upon which he relies. While admitting that the larvae of *Contracaecum*, as they occur in fishes, can usually be identified generically, the writer has very great doubts regarding the possibility of their specific determination, and considers that much comparative morphological work is still necessary before such a claim can be established for any of these genera.

SUMMARY

Reasons are given for rejecting a recent proposal to change the names *Anisakis simplex* to *Capsularia marina*, *Anisakis* to *Capsularia*, Anisakinae to Capsulariinae and *Porrocaecum decipiens* to *P. piscium*. The question of the possibility of identifying the larval forms of Ascaridae occurring in fishes is discussed.

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OBSERVATIONS ON THE NEMATODE *MERMIS NIGRESCENS* AND RELATED SPECIES

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(With 17 Figures in the Text)

The sight of a slender worm, looking like an animated length of thread and slowly waving part of its body about in the air, on a leaf or stem of some plant, perhaps at a height of 2 or 3 ft. from the ground, is a constant source of wonder to gardeners and others who experience it for the first time. *Mermis nigrescens* Dujardin, 1842, differs from the majority of the Nematodes of the family Mermithidae in that the female worm has this habit of climbing plants to lay her eggs. The Mermithidae in general, after a larval period spent as parasites in the body-cavity of insects or other invertebrates, pass the rest of their lives either in water or buried in the soil, the females laying their eggs there and leaving them to hatch. The larvae then wander about and, on meeting with a suitable host, bore their way into it by their own exertions. Dujardin, whose specimens of *M. nigrescens* were found in gardens at Rennes in France, suspected that it was a parasite of the larvae of cockchafers or other insects, from which it emerged to lay its eggs. In this surmise he was considerably nearer to the truth than those observers who have put forward stories of 'showers of worms', but he did not know, as we do now, that between the emergence of the parasite from its host and its appearance above ground as an adult worm a period of two years usually elapses, during which it lives buried at a considerable depth in the soil and slowly uses up the food-material derived from the host in the elaboration of its sexual organs and its eggs.

When fully mature and ready to lay her eggs, the female comes up out of the ground under suitable conditions—usually after a thunderstorm—and climbs on to plants, on which the eggs are deposited wherever she crawls. The eggs themselves, in this species, are specially adapted for this mode of dissemination. They have a thick, brownish inner shell, highly resistant to drying, and a thinner outer membrane provided at two opposite poles with branching filaments, probably of an albuminous nature, which assist in their attachment to the surface of plants, and also to some extent keep them together in small clumps. These eggs do not hatch until they are swallowed by a suitable phytophagous insect, when the larvae penetrate the wall of the insect's gut and enter its body-cavity, and there proceed to grow rapidly to the full size of the adult worm.

The foundations of our present knowledge of the habits and life-history of *M. nigrescens* were laid by v. Siebold (1850) and extended by Hagmeier (1912). The accounts given by European workers have been confirmed and elaborated by the work of American observers (Cobb, Steiner & Christie, 1925; Cobb, 1926; Christie, 1937), who have worked on a form which, in the writer's opinion, is probably identical with *M. nigrescens*, but which Cobb (1926) separated from it as a distinct species under the name of *M. subnigrescens*. No evidence has been observed of the existence in Great Britain of more than one species having the peculiar habits of the worm here called *M. nigrescens*, and it is assumed that this species is that originally described by Dujardin. Cobb not only regarded the American form as distinct, but also questioned the identity of the European forms called *M. nigrescens* by other authors (Meissner, 1856; Hagmeier, 1912). The arguments on which Cobb's views were based appear to be of a very slender character and in part self-contradictory, as when he argues that the eggs are 'globular' in *M. subnigrescens*, as distinct from the 'lenticular' eggs described by Meissner, but elsewhere in the same paper describes them as 'subspherical' and having 'somewhat the form of an ellipsoid of revolution'. Cobb's case seems to be further weakened by his suggestion that the male worms described by Hagmeier in Germany may have belonged not to *M. nigrescens* but to the American species *subnigrescens*. In any case, it is perhaps sufficient to point out that at the end of his paper Cobb remarks that 'only further research can determine whether the names *nigrescens*, *meissneri* and *subnigrescens* should denominate specific, varietal, or, conceivably, only racial differences'.

OCCURRENCE IN THE BRITISH ISLES

A number of inquiries, usually accompanied by specimens, are received every summer at the British

Museum (Natural History) about worms having the habits described above for the female of *M. nigrescens*. No systematic survey has been carried out to determine the distribution of the worm, but these casual records have been collected for a period of about 22 years, and an analysis of them shows that the worm is very generally distributed in these islands, occurring from the north of Scotland to the south of England.

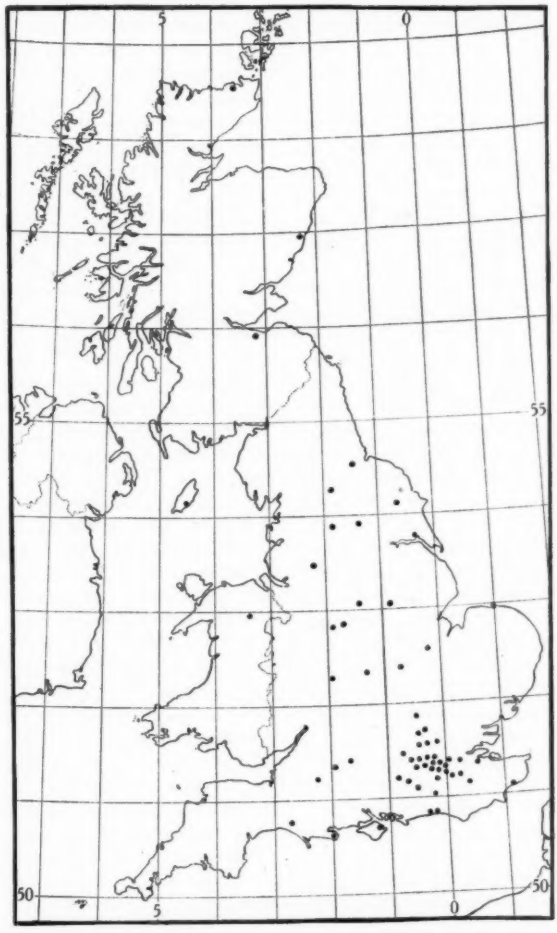

Fig. 1. Map of the British Isles, showing the approximate localities in England, Wales and Scotland from which records of *Mermis nigrescens* have been obtained.

The accompanying map (Fig. 1) shows the approximate localities from which evidence of the occurrence of *M. nigrescens* has been obtained. On a map of this size it is impossible to indicate every locality by a separate dot, as in places the dots would be so closely crowded as to overlap. It will be noticed that there is a specially heavy concentration on the outskirts of London and in the home counties. This does not necessarily mean that the worm occurs more commonly there than in other parts of the country, but is probably due mainly to two factors: (1) the concentration in this area of numbers of small suburban gardens, where the worm is more likely to be observed than elsewhere; and (2) the fact that the Museum is better known to the population of greater London than to dwellers in the provinces, and therefore inquiries are most frequently received from this area. *M. nigrescens* probably occurs just as abundantly in suitable parts

of the open country (heaths, downs and meadows, especially where grasshoppers occur) as in suburban areas, but naturally is more rarely observed there. There is, however, a significance in its frequency in relatively built-up areas, where grasshoppers are scarce or absent, to which further reference will be made below.

A single record received from Northern Ireland was not accompanied by precise data. Southern (1914) has recorded the worm from Ireland (Co. Mayo).

SEASONAL APPEARANCE

The meteorological condition that invariably precedes the appearance of adult females of *M. nigrescens* above ground is a heavy downpour of rain, and it is usually after such downpours in the summer, commonly accompanying thunderstorms, that the worms are observed—sometimes in such numbers as to have suggested the idea that they have descended from above with the rain. If gravid female worms are buried at some depth in fairly dry soil in a glass jar, and a quantity of water is suddenly poured on to the surface of the soil, the worms can be induced to come to the surface and begin climbing up the sides of the jar. Christie (1937) describes an experiment in which, by watering a plot of ground with a lawn-sprinkler for 3½ hr. in the evening, female *Mermis* were induced to come to the surface, and were found laying their eggs the following morning.

The worms can, naturally, climb only on a wet surface. Hence it is usually early in the morning, before the heat of the sun has dried up the moisture, or soon after a heavy shower later in the day, that they are found on plants. If the foliage becomes dry the worms coil up and fall to the ground. It has been shown by Cobb (1926, 1929) that oviposition takes place only under the influence of light and a certain amount of radiant heat. He found (1926) that oviposition was 'gradually stopped by yellowish green glass known to cut out about 80% of the heat of direct sunlight and by foliage (e.g. grape leaf) of similar color', and suggests that this inhibition by 'foliage green' would ensure the deposition of the eggs above the green shadows of the habitat, and therefore where they would have the best chance of being swallowed by grasshoppers.

The time of year at which the female worms first begin to leave the soil probably varies with latitude, temperature and other conditions besides moisture. Dujardin (1842, 1845) obtained them in March, April, May and June at Rennes, France. At Woods Hole, Massachusetts, Cobb mentions oviposition in April or early May, but Christie found that it usually began towards the end of May and continued until some time in August. In Germany, gravid females are recorded in June at Munich and Göttingen (Meissner, 1856) and at Heidelberg (Hagmeier, 1912).

Many of the inquirers in this country who send specimens to the British Museum fail to give a precise date of collection, but of ninety-one records, the dates of which were given, or could be determined within a day or two, sixteen occurred in May, fifty-six in June and nineteen in July. In one instance the worm seems to have been observed on or about 12 May, and this is the earliest record received. The next earliest is on or about 22 May. Thus the *Mermis* season, which usually coincides with a period of frequent thunderstorms, is generally at its height about the middle of June, and continues, with a gradual falling-off in the number of records, until the middle of July. Only two occurrences have been noted later than 25 July, the latest being 28 July. There is therefore little evidence in favour of the view that worms that fail to deposit all their eggs at the first opportunity may retire again into the soil and repeat the process at a later date in the same season.

HOSTS

Dujardin, as we have seen, guessed that cockchafers or other insects were the hosts of *M. nigrescens*. Diesing (1851) quotes 'Audin'* as authority for mentioning *Melolontha vulgaris* (the common cockchafer) as one of the hosts of the worm, and gives also a list (compiled from previous authors) of seven other insect hosts, five of which are Orthoptera and two Lepidoptera. Since that time a large number of invertebrates have been cited as hosts, but it may be doubted in many cases whether the specific determination of the worm was correct. There has been much confusion in the literature, not only between *M. nigrescens*, *M. albicans* and other Mermithidae, but also between the Mermithidae and the Nematomorpha (Gordiids). Moreover, the specific determination of Mermithidae in the larval stage is a matter of great difficulty, and may be impossible unless they are reared to maturity. Hence the definite assignment of the parasitic phase to a particular species without experimental proof must always be regarded with some suspicion.

Almost all the hosts cited for *M. nigrescens* are insects, and most of these belong to the order Orthoptera. So far as number of species is concerned, grasshoppers appear to be by far the most important.† A few insects of other orders have also

* The reference is apparently to Audouin, who found '*Gordius*' in the larvae of the cockchafer—see Leblond, 1837, *Nouvel Atlas* [to the French edition of Bremser, *Traité...sur les Vers Intestinaux de l'Homme*], p. 57. Dujardin (1842, p. 134) assumed these to be *M. nigrescens*.

† See the lists of hosts given by v. Linstow (1878, 1892, 1898).

been mentioned, but most of these records seem to require confirmation, while such hosts as a slug and a turbellarian worm (both quoted by v. Linstow (1878)) seem even more improbable.

The first experimental evidence that grasshoppers can serve as hosts for *M. nigrescens* seems to have been given by v. Siebold (1850). He obtained larvae from *Tettigonia verrucivora* [*Decticus verrucivorus*], *Phasgonura* [*Locusta*] *viridissima* and *Chorthippus scalaris* [*Gomphocerus morio*] near Freiburg in the late summer, and allowed them to bury themselves in soil in flowerpots. On 24 July of the following year he was able to identify the worms as *Mermis nigrescens*, since they already contained eggs of the typical form, with appendages. Hagmeier (1912) also found parasitic larvae, from which he was able to rear adult forms of *M. nigrescens*, in naturally-infested grasshoppers (various unnamed species of *Stenobothrus* and *Decticus*), and was the first observer to discover the male worm. He found natural infestation in no host other than grasshoppers, and his attempts to infect 'caterpillars and grubs' [Raupen und Engerlinge*] experimentally were without result.

The American form of the worm has been recorded as occurring, naturally or experimentally, in a considerable number of species of grasshoppers, of which a list is given by Christie (1937, p. 362), but apparently in no other host.

In view of the frequency with which specimens of *M. nigrescens* appear in suburban gardens near London, in which grasshoppers are probably absent or at least rare, the writer has always felt that some other common invertebrate probably served as their host. During the past 14 years a number of attempts have been made, as opportunity offered, to obtain experimental evidence on this point. To give details of all the experiments would be tedious, especially as most of them gave entirely negative results.

Feeding experiments with eggs of *M. nigrescens* have been carried out on the following insects:

LEPIDOPTERA. Larvae of *Abraxas grossulariata*, *Cerura vinula*, *Hyponomeuta euonymella*, *Malacosoma castrensis*, *Mamestra brassicae*, *Smerinthus ocellatus*, *Smerinthus populi*, *Taeniocampa stabilis*, *Tortrix heparana*.

HYMENOPTERA. Larvae of *Trichiocampus viminalis*.

ORTHOPTERA. Hoppers of undetermined species of grasshoppers; hoppers of *Locusta migratoria*.

DERMAPTERA. Nymphs of *Forficula auricularia*.

Attempts have also been made to infect the following molluscs (slugs): *Agriolimax agrestis*, *A. agrestis pallida*, *Arion hortensis*, *Milax sowerbyi*.

The usual procedure in all these experiments was to spread a suspension of eggs (usually naturally deposited, but sometimes teased out of the uterus of gravid worms) in water on the food by means of a camel-hair brush. In some cases repeated infective feeds were given, to ensure as far as possible that some of the eggs had been ingested.

MOLLUSCA. The slugs gave entirely negative results. Several of them lived only 13–20 days after the first infective feed; some were apparently eaten by their companions, and the remainder were killed on the 48th and 49th days of the experiment. In none of them, on dissection, were any *Mermis* larvae found.

LEPIDOPTERA. Most of the Lepidoptera also proved negative. As a rule the larvae were allowed to pupate, and the moths examined for *Mermis* when they emerged. Any larvae that died before pupation were dissected. In one of two larvae of *Smerinthus ocellatus* that died 5 days after the infective feed, numerous unhatched eggs of *Mermis* were found in the intestine. In other larvae that survived longer nothing was found. Two of a batch of ten larvae of *Malacosoma castrensis* died 18 days after feeding. On dissection, two small *Mermis* larvae were found in the body cavity of one of them. These measured 2·47 and 2·63 mm. in length, and still showed the mouth-stylet of the newly-hatched larva. In another larva that died 27 days after feeding, five similar larvae were found. These were dead, brown and surrounded by masses of adventitious tissue (probably 'phagocyted'*). Such 'phagocyted' larvae were also found in three out of five more larvae which were killed and dissected 5 days later. Of a large number of larvae of *Hyponomeuta euonymella*, which pupated almost immediately after being given an infective feed, it was doubtful whether any had ingested eggs. The 'fed' moths that emerged after 17–34 days all gave negative results, but two of the controls, dissected on the 20th day of the experiment, contained each one small unidentified Nematode larva.

HYMENOPTERA. Several larvae of the poplar sawfly (*Trichiocampus viminalis*) were given an infective feed on 8 July 1930. Four imagos that had emerged about 6 or 7 August were found dead on 11 August and dissected. One was negative, but in the others four, seven and twenty-three larval worms respectively were found in the thoracic and abdominal cavities. These larvae varied in length from 2·9 to 6·7 mm. Another imago was found dead on 12 August, and contained seven larvae measuring 1·65–5·4 mm. It seems, therefore, that some development of the worms had occurred in this host. The larvae had migrated to the body-cavity, and in 34–35 days some of them had grown to a length of over 5 mm. But *T. viminalis* is so

* The term Engerlinge, as I am informed by Dr F. I. Van Emden, is applied in Germany particularly to the larvae of lamellicorn beetles, including cockchafers.

* See W. R. Thompson, 1913, *C.R. Soc. Biol., Paris*, **75**, 559; and 1915, *Bull. Soc. Zool. Fr.* **40**, 63.

small an insect, and its metamorphosis is completed in so short a time, that the worm would probably be unable to develop in it to the stage at which it normally leaves the host.

ORTHOPTERA. The single experiment carried out on grasshoppers had, rather curiously, a negative result. Only seven hoppers, however, were fed with eggs, and it is possible that they had ingested none of them, as six of them died within 10 days, perhaps owing to unsuitable conditions. The seventh survived about 6 weeks, but was not dissected, as it was found in a dried-up condition on the writer's return from a temporary absence.

In 1933–8 experiments on the breeding of locusts were being carried out in the Museum by the Imperial Institute of Entomology, and a large stock of these insects was maintained under the charge of Dr A. G. Hamilton. On several occasions Dr Hamilton found young or adult locusts (*Locusta migratoria migratorioides*) dead, apparently as the result of infection with mermithid worms. The latter were all female larvae, and varied in length from 106 to 155 mm. Similar worms, 168–172 mm. long, were found in *Schistocerca gregaria*. As all the locusts had been bred from eggs, it seemed clear that the infection must have been introduced with the food (mainly grass) supplied to them, and that the worm with which they had thus become accidentally infested must be of British origin. *Mermis nigrescens* was naturally suspected, and attempts were made by Dr Hamilton, at the writer's suggestion, to keep some of the larvae alive until they reached the adult stage. These attempts, unfortunately, were unsuccessful.

It was also thought worth while to try to infect some of the locusts experimentally with known *M. nigrescens*. Accordingly, eggs that had been naturally laid on grass were given to five 2nd instar hoppers of *Locusta migratoria* on 23–25 June 1937. It may be mentioned that the locusts were kept at a temperature of 90° F. during the day, and at the ordinary temperature of the laboratory at night. On 5 July two of the hoppers were found dead, but as they were considerably decomposed, were not dissected. On 7 July another was found in a half-moulted condition, and unable to complete the moult because of the escape of body-fluid from its head. It was killed and dissected, and 106 small *Mermis* larvae were found in the cavities of its head, thorax and abdomen, but none in its gut. The remaining two hoppers died, unable to moult, on 9 and 12 July, and were found on dissection to contain respectively 172 and 234 larvae. Another hopper of the same species was given a feed of *Mermis* eggs on 12 July, and was found dead on 20 July. It contained forty-six very young larvae.

This experiment would therefore, in all probability, have led to the successful development of the worms had it not ended prematurely. It affords a striking example of the dire results that may follow excessively heavy infection, even in suitable hosts.

The writer has, on several occasions, received specimens of two types of immature Mermithidae found in wild locusts in various parts of Africa. One form has a sharply pointed caudal appendage, while the other is blunt-tailed and closely resembles *M. nigrescens*. In view of the possibility of locusts being infected with *M. nigrescens*, the specimens of the blunt-tailed form have been re-examined in order to see whether they might, in fact, belong to that species. The hosts from which these blunt-tailed larvae were obtained were the African migratory locust (*Locusta migratoria migratorioides*) in Kenya, Tanganyika, the Belgian Congo, South-west Africa, the Gold Coast and Sierra Leone; the desert locust (*Schistocerca gregaria*) in Tanganyika; the red locust (*Nomadacris septemfasciata*) in Southern Rhodesia; and an unnamed locust in Uganda. All the worms whose sex could be distinguished appeared to be females, and no definite morphological difference could be found between these larvae (Fig. 6), those obtained from locusts in captivity by Dr Hamilton, and those of a similar stage from other hosts, known or believed to be *Mermis nigrescens* (Figs. 2–5). The African specimens, however, attain a much larger size than is usual for *M. nigrescens* in Europe (or its counterpart in North America). Individuals 200 mm. or more in length appear to be quite common. One from *Locusta migratoria* in Uganda measured 280 mm., while the single specimen from *Nomadacris septemfasciata* measured no less than 450 mm.

According to Lopez (1933) one of two species of mermithids found in *Locusta migratoria* in the island of Negros, Philippines, was determined by Steiner as *Mermis subnigrescens*. The lengths of three specimens are given as 43·2, 28·8 and 35·3 cm., but it is not clear to which of the two species these belonged. A form named *M. longissimus* by Fedchenko (1874) was found emerging from a locust (doubtless *Locusta m. migratoria*) in Turkestan. It measured 440 mm. in length.

It is, of course, possible that *Mermis nigrescens* may grow to an unusually large size in locusts, perhaps as a result of the abundant supply of food in the body-cavity of so large an insect, and this may be especially the case in tropical and subtropical latitudes. There is, however, so far as the writer is aware, no evidence at present of the occurrence of the adult form of *M. nigrescens* in Africa, though a very closely related but evidently distinct species from Mt Kenya will be described later in this paper.

DERMAPTERA. The most striking experimental results were obtained with earwigs (*Forficula auri-*

cularia). This insect is cited as a host for *Mermis nigrescens* by v. Linstow (1878), but on what evidence is not stated. The writer had on seven occasions received specimens of immature mermithids found in, or emerging from, earwigs in England. In view, however, of the difficulty of specific determination at this stage, it was not considered safe to assume that these larvae belonged to *M. nigrescens*.

In June 1943 feeding experiments on earwigs were started. On 23 June one earwig nymph was fed with a young rose-leaf, on the petiole of which were several Aphid nymphs, and which had been spread with a suspension of the eggs of *M. nigrescens* in water. The eggs were distributed over the upper surfaces of the leaflets and on the petiole, and care was taken to make sure (under a lens) that some were placed on the backs of at least some of the aphids. During the night most of the aphids and part of a leaflet of the rose-leaf appeared to have been eaten. The rest of the leaf was left in the tin box containing the earwig until 26 June, but there was no evidence that any more of it was eaten. This earwig was found dead on 6 July (having been apparently healthy and feeding well in the interval), and on dissection was found to have about sixty-five larval worms in its body-cavity, varying in length from 0·8 to 1·5 mm. There was also one larva of much greater size (4·3 mm.), which may have represented a previous natural infection.

Another earwig was fed with the remains of the same rose-leaf on 25–28 June. This insect was found dead on 16 July, possibly as the result of some injury from the accidental knocking over of its box a day or two previously, when it was moulting. On dissection it was found to contain two larval worms measuring 2·3 and 2·6 mm. in length.

On 25 June a batch of fifteen earwigs was divided into two lots, the smaller lot of six being kept as controls. The other nine were given an infective feed, the eggs being spread partly on two small rose-leaves, with aphids, and partly on a small piece of lettuce-leaf. During the night many of the aphids appeared to have been eaten, and also part of the lettuce-leaf. In the morning more *Mermis* eggs were applied to the few aphids that remained, and these were almost all eaten by the morning of the following day. The remains of the infective meal were all removed on the 29th, and the earwigs were then fed daily on a diet consisting mainly of small pieces of lettuce, supplemented every 3 or 4 days with a little animal food, usually in the form of blowfly pupae previously cut open. Seven of these nine earwigs subsequently proved to be infested with *Mermis* in varying numbers. Two of them died prematurely, evidently as the result of very heavy infections, the first on 17 July, with about 120 larvae varying in length from 1·9 to 5·6 mm., and the second on 29 July, with about eighty-seven larvae, 6·5–25 mm. long. Four earwigs lived until various dates in August, when full-sized worms emerged naturally from them. The three remaining were kept alive until 4 October, when they were killed and dissected. Of these one was found to contain a single worm (the largest obtained), and two were free from parasites.

Full-sized worms emerged from three of the six control earwigs in August and September; the other three were killed on 4 October, and were all free from parasites.

The results of these experiments are summarized in the table on p. 129. Several points of interest may be noted:

(1) It seems impossible to doubt that at least the first four earwigs shown in the table, which had heavy infections, had acquired these as the result of the experimental feeding.

(2) In view of the fact that half of the controls evidently harboured natural infections, it is possible that some of the lighter infections in the 'fed' series may also have been naturally acquired before the experiment.

(3) It is evident that the common earwig is a suitable host for *Mermis nigrescens*, and that the worm is able to complete its growth and development in this host up to the point of normal emergence. There can be little doubt that the earwig is very commonly infested in nature, and that, owing to its ubiquity, it is an important host for the species.

(4) It is to be observed that, where the parasites had reached a stage at which their sex could be distinguished, if the infection was relatively heavy (thirteen worms or more per host) they were all males; whereas in the lighter infections (five worms or less) they were all females.* This is in agreement with the findings of Cobb *et al.* (1927) and Christie (1929) for the American form in grasshoppers.

(5) The measurements of the larvae at different ages show their extremely rapid rate of growth. It is also evident that females remain much longer in the host than males. Males emerged after 4–5 weeks, while no females appeared until 6 weeks after the experimental feeding, and some of these may have been already present as natural infections. One female had not yet emerged by the 14th week. This is in accordance with the findings of American observers in grasshoppers.

* Some of the female worms (Fig. 5) showed a suggestion of the rudiment of the male genital pore, as has been described by Steiner (1923) for *Agamermis decaudata*. They may therefore be regarded as intersexes, suggesting that the potentiality to develop into an adult of either sex is present in every individual, and that it depends on external conditions (probably mainly on the available supply of food-material) which sex predominates.

(6) Very heavy infections appear to cause the death of the host within the first 2–4 weeks. It was also observed (as in the American experiments) that if the host dies before the emergence of the worms, the latter also die. In multiple infections, if the host dies after the emergence of one or two worms, the worms which have failed to emerge perish.

(7) The host invariably died soon after the emergence of the parasites. This might occur immediately, or the host might appear quite lively for a time, and be found dead from 24 to 48 hr. later. How long the host survives probably depends on the extent of the 'bleeding', or exudation of body-fluid. The majority of the worms that emerged appeared to have done so by way of the anus of the host, having probably pierced the wall of the rectum. In these cases some soil was usually found adhering to the anal region of the earwig. One of the experimental earwigs was found in a moribund condition, with part of a worm protruding from its mouth. Dissection appeared to show that the worm had crept forward through the thoracic region dorsally to the oesophagus and entered the buccal cavity from the dorsal side. A worm from a naturally-infested earwig was also received from a correspondent with the observation that it had been found emerging from the mouth.

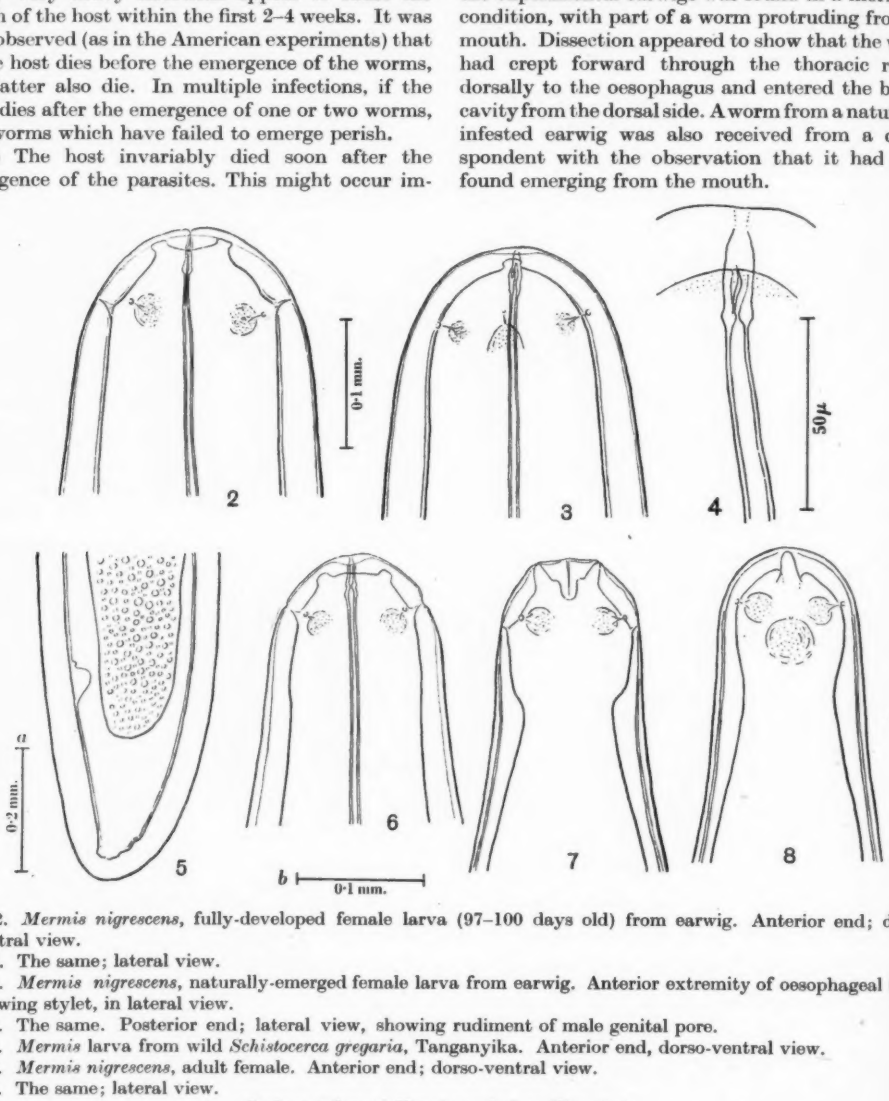

Fig. 2. *Mermis nigrescens*, fully-developed female larva (97–100 days old) from earwig. Anterior end; dorso-ventral view.
Fig. 3. The same; lateral view.
Fig. 4. *Mermis nigrescens*, naturally-emerged female larva from earwig. Anterior extremity of oesophageal tube, showing stylet, in lateral view.
Fig. 5. The same. Posterior end; lateral view, showing rudiment of male genital pore.
Fig. 6. *Mermis* larva from wild *Schistocerca gregaria*, Tanganyika. Anterior end, dorso-ventral view.
Fig. 7. *Mermis nigrescens*, adult female. Anterior end; dorso-ventral view.
Fig. 8. The same; lateral view.

Scale *a* refers to Fig. 5; scale *b* to Figs. 6–8.

It had been hoped to rear some of the emerged worms to maturity, but unfortunately none of them survived more than a few weeks. Special boxes of plaster-of-Paris were made and filled with soil, and in these the worms were placed, the intention being to bury the boxes later in the autumn, under as nearly as possible natural conditions, out of doors. The mortality among the worms appeared to be due to two causes. They were frequently bitten, sometimes

into fragments, or partly eaten, by the earwigs, and the slightest injury at the time of emergence was invariably fatal. Even if not so severely injured that they died at once, they were always attacked by a fungus which rapidly spread its mycelium throughout the body and absorbed the food-material stored in the 'fat-body'. Even specimens that appeared to be quite uninjured and healthy were, sooner or later, and in spite of every effort to keep them under 'sterile' conditions, attacked by this fungus, and ultimately succumbed.

It had been intended to include in this paper some account of the larval development of *M. nigrescens*, and in particular of the development of the so-called 'oesophagus' and the structures connected with it, but the material at present available does not appear to the writer to warrant sufficiently definite conclusions. Figures are given of certain features of the late larval stage, at the time of emergence from the host (Figs. 2–6). The head shows the same number and arrangement of sense-organs as in the adult, namely a pair of lateral papillae near the mouth, and a circle of six nerve-endings (two lateral and four submedian) further back. Attention may be drawn to one small anatomical detail. Linstow (1892, Pl. XXVIII, Fig. 1 *c*) has figured a spindle-shaped enlargement of the anterior end of the cuticular oesophageal tube, containing what appears to be a forwardly-directed and sharply pointed tooth or stylet. He says nothing about this in the text, but it appears to be regularly present in the writer's material at this stage (see Figs. 2, 3, 4, 6).

Earwig	Date of experimental feeding	Date of emergence of worms	Date of death of earwig	No. of worms	Sex of worms	Approximate length of worms (mm.)	Probable age of worms (days)
1	23–25 June	—	6 July	65	—	0·8–1·5 [one 4·3]	11– 13
2	25–28 June	—	16 July	2	—	2·3, 2·6	18– 21
3	,,	—	17 July	120	—	1·9– 5·6	19– 22
4	,,	—	29 July	87	♂♂ or unknown	6·5–25	31– 34
5	,,	1 Aug.	1 Aug.	13 (2 emerged)	♂♂	38·5–50	35– 37
6	,,	1 Aug.	1 Aug.	15 (2 emerged)	♂♂	26·5–38	35– 37
7	,,	8–9 Aug.	9 Aug.	5 (?)	♀♀	45 [only one complete]	42– 45
8	,,	22 Aug.	23 Aug.	1	♀	125	55– 58
9	,,	—	Killed 4 Oct.	1	♀	130	97–100
10	,,	—	Killed 4 Oct.	—	—	—	—
11	,,	—	Killed 4 Oct.	—	—	—	—
				Controls			
12	—	12 Aug.	12 Aug.	3 (?)	♀♀	55, 75 [one in fragments]	—
13	—	22 Aug.	22 Aug.	1	♀	[damaged]	—
14	—	18 Sept.	20 Sept.	1	♀	77	—
15	—	—	Killed 4 Oct.	—	—	—	—
16	—	—	Killed 4 Oct.	—	—	—	—
17	—	—	Killed 4 Oct.	—	—	—	—

Brief descriptions may now be given of two apparently new species of *Mermis*, evidently very closely related to *M. nigrescens*.

Mermis kenyensis n.sp.

(Figs. 9–12)

A single adult female specimen. Mt Kenya, 1927 (A. Insole *leg.*).

The worm measures about 92 mm. in length, and has a maximum thickness of 0·35 mm. The diameter near the anterior end, at the level of the lateral organs, is about 0·1 mm. The cuticle is extremely thick, especially near the extremities. Its thickness varies from 32 to 50 μ in different regions,* and it consists of the same layers, including the criss-cross fibrils, as in *M. nigrescens*. There is little in the characters of the anterior extremity (Figs. 9, 10)

* In adult females of *M. nigrescens* the general thickness of the cuticle, apart from local thickenings near the extremities, was found to vary from about 14 to 20 μ. According to Meissner (1856), however, the inner layer ('corium') alone is $\frac{1}{50}$–$\frac{1}{60}$‴ thick [23·5–26·5 μ].

to distinguish this species from *M. nigrescens* (Figs. 7, 8), except perhaps the rather smaller size of the oral papillae and the rather more forward position of the lateral organs. The mouth is very inconspicuous, and shows no special thickening of the cuticle. Of the œsophageal tube nothing could be made out. The nerve-ring is situated at about 0·4 mm. from the anterior end. In the preserved specimen no pigment is visible behind the head.

The caudal end (Fig. 11) is blunt, as in *M. nigrescens*, with a slight ventral curvature. The rudiment of the male genital pore is represented by a little depression in the surface of the cuticle at about 0·3 mm. from the extremity.

The vulva is an inconspicuous longitudinal slit situated at about 50 mm. from the anterior end (i.e. slightly behind the middle of the body). There is a short muscular vagina, but its form could not be made out owing to the dense mass of eggs in the uterus.

The eggs (Fig. 12), as in *M. nigrescens*, have the form of somewhat flattened spheres. They have a relatively thin outer coat, probably of albuminous material, which appears smooth and is without local thickenings or appendages, and apparently without an equatorial suture; and a thick, brownish inner shell like that of *M. nigrescens*. The outside measurements of the eggs are: longer diameter 44–47 μ; shorter diameter 31–32 μ. The measurements of the inner shell are: longer diameter 42–43 μ; shorter diameter 29–30 μ. The embryos are fully formed *in utero*.

Steiner (1921) has described a very similar form, from the Pacific island of New Ireland (Neu Mecklenburg), under the name of *Mermis nigrescens* Dujardin, var. *athysanota*. In the eggs of this form, however, the outer coat seems to be thicker than the inner shell, and to have an irregular, almost warty, surface. Though both this and the form described above are clearly very closely related to *M. nigrescens*, the writer feels that they should be regarded as distinct species,* and that Steiner's form should be called *Mermis athysanota* (Steiner, 1921).

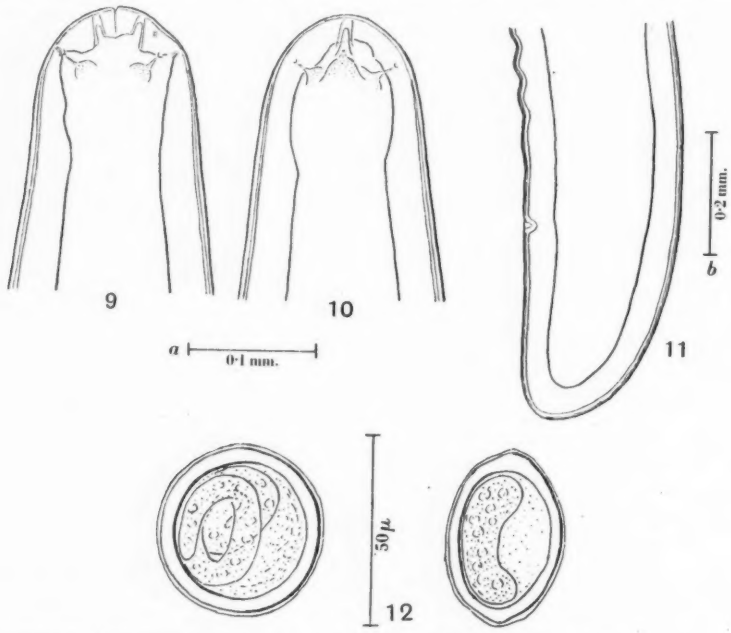

Fig. 9. *Mermis kenyensis*, adult female. Anterior end; dorso-ventral view.
Fig. 10. The same; lateral view.
Fig. 11. The same. Posterior end; lateral view, showing rudiment of male genital pore.
Fig. 12. *Mermis kenyensis*. Eggs, viewed from two aspects.

Scale *a* refers to Figs. 9 and 10; scale *b* to Fig. 11.

* They differ from *M. nigrescens* and from each other much more conspicuously than does '*M. subnigrescens*' from the typical form.

Mermis tahitiensis, n.sp.

(Figs. 13–17)

A single adult female specimen. Papeari, Tahiti, Society Islands, March 1928 (Harrison W. Smith *leg.*).

The total length is 49·5 mm., and the maximum thickness 0·25 mm. The diameter near the anterior end, at the level of the lateral organs, is 0·094 mm. cuticular walls. An oesophageal tube could not be made out. The nerve-ring is situated at 0·27 mm. from the anterior end. In the preserved specimen no pigment is visible behind the head.

The caudal end (Fig. 15) is bluntly conical and not quite so rounded as is usual in *M. nigrescens*. At 0·26 mm. from the extremity there is a very slight protuberance of the cuticle, perhaps representing a rudimentary male genital pore.

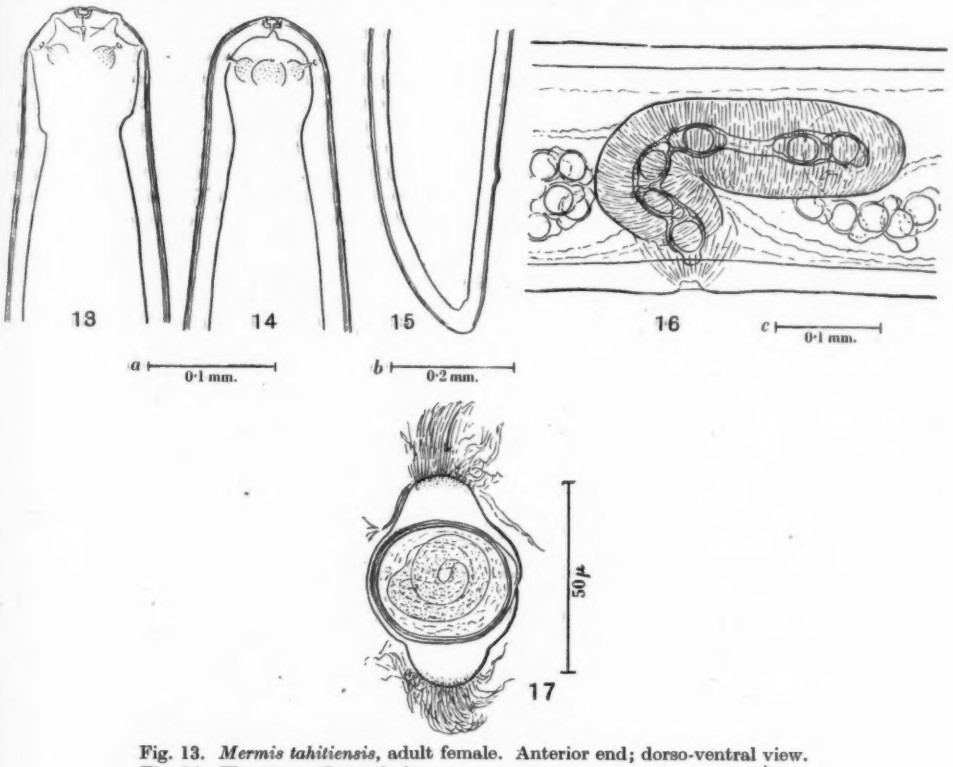

Fig. 13. *Mermis tahitiensis*, adult female. Anterior end; dorso-ventral view.
Fig. 14. The same. Lateral view.
Fig. 15. The same. Posterior end; lateral view.
Fig. 16. The same. Portion of middle region, showing vulva and vagina.
Fig. 17. *Mermis tahitiensis*. Egg.

Scale *a* refers to Figs. 13 and 14; scale *b* to Fig. 15; scale *c* to Fig. 16.

Apart from some thickening near the extremities, the cuticle is of almost uniform thickness (20–24 μ) throughout. It consists of the same layers as in *M. nigrescens*, the inner layer ('corium') having, as usual, a vertically striated appearance in optical section, and the two layers of oblique fibrils being also present below the outermost layer. The characters of the anterior end (Figs. 13, 14) are very similar to those in *M. nigrescens*, but there is a small, straight-sided buccal cavity with thickened

The vulva is situated slightly behind the middle of the body, at 29 mm. from the anterior end. There is a short, stout, somewhat C-shaped muscular vagina (Fig. 16), and the two opposed branches of the uterus form, as usual, a continuous tube. The eggs (Fig. 17) are somewhat reminiscent of those of *M. nigrescens*, but the polar knobs of the outer coat are of relatively enormous size, and the inner shell is thin and colourless. The eggs are very uniform in size. Their length (including the polar knobs) is

about 54–56μ. They are slightly flattened, the longer transverse diameter being 40μ, the shorter 28μ. The outer coat is thin at the equator, where it breaks easily under slight pressure into two cup-shaped halves, but there does not seem to be a definite equatorial groove or suture, as in *M. nigrescens*. At the summits of the polar knobs there are usually at least some irregular strands of the (presumably) albuminous material that forms the outer coat, and frequently there is a 'byssus' or bunch of fine threads, as indicated in the figure. This has quite a different appearance, however, from the relatively stout threads, frayed out into tassels at the ends, characteristic of *M. nigrescens*. The inner shell is slightly ovoid, its longer diameter being at right angles to the long axis of the outer coat. It measures about 36–38 × 30–32μ. The embryos are fully formed *in utero*.

SUMMARY

The main features of the life-history of *Mermis nigrescens*, so far as it is known, are briefly discussed. Some data have been collected on its distribution in the British Isles, and on the seasonal appearance of the egg-laying females. The insect hosts of the worm are discussed, and feeding experiments on various invertebrates are recorded, including the experimental infection of the common earwig (*Forficula auricularia*). This insect, which has also been found naturally infested, is regarded as an important host for the species, more especially where grasshoppers are absent. Observations by other workers on the influence of the number of larvae per host on the sex of the developing worms, and on the survival of the host, have been confirmed. Two new species, closely related to *M. nigrescens*, are described: *M. kenyensis* and *M. tahitiensis*, from Africa and Tahiti respectively.

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